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(54) Title: NOVEL 3-NITROPYRIDINE DERIVATIVES AND THE PHARMACEUTICAL COMPOSITIONS CONTAINING SAID DERIVATIVES

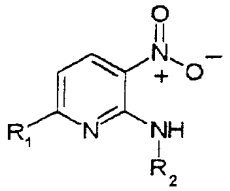
(57) Abstract: The present invention relates to novel 3-nitropyridine derivatives and the pharmaceutical compositions containing said derivatives, and more specifically, to 3-nitropyridine derivatives and their pharmaceutically acceptable salts, the process for preparing them, and the pharmaceutical compositions containing said compounds as active ingredients. In particular, said 3-nitropyridine derivatives of the present invention, due to their inhibitory activity against the proliferation of human immunodeficiency virus (HIV) as well as hepatitis B virus (HBV), can be used as a therapeutic agent as well as a preventive agent for hepatitis B and acquired immune deficiency syndrome (AIDS).

NOVEL 3-NITROPYRIDINE DERIVATIVES AND THE PHARMACEUTICAL COMPOSITIONS CONTANING SAID DERIVATIVES

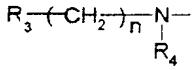
TECHNICAL FIELD

The present invention relates to novel 3-nitropyridine derivatives and the pharmaceutical compositions containing said derivatives. More specifically, the present invention relates to 3-nitropyridine derivatives and their pharmaceutically acceptable salts, represented by the following formula 1, which effectively inhibit proliferation of hepatitis B virus and human immunodeficiency virus. This invention also relates to the process for preparing 3-nitropyridine derivatives and to the pharmaceutical compositions containing said derivatives as effective ingredients against viruses.

Formula 1



Wherein,

R_1 is methoxy or  ;

R_3 is H, hydroxy, dialkylamino group with $C_2 \sim C_6$, straight or branched hydroxyalkyl group with $C_2 \sim C_6$, straight or branched dihydroxyalkyl group with $C_3 \sim C_6$, alkoxyalkyl group with $C_3 \sim C_6$, or saturated or unsaturated 5 or 6 membered heterocyclic compounds containing 1 to 3 heteroatoms selected from N, O, and S, which may be unsubstituted or substituted with alkyl group of $C_1 \sim C_3$; R_3 may or may not contain asymmetrical carbons;

R_4 is H, straight or branched alkyl group with $C_1 \sim C_4$, or cycloalkyl group with $C_3 \sim C_6$;

R_3 and R_4 both may consist of 5 or 6 membered heterocyclic ring containing $1 \sim 3$ heteroatoms selected from N, O, and S, which is unsubstituted or substituted with straight or branched alkyl group with $C_1 \sim C_5$, straight or branched hydroxyalkyl group with $C_2 \sim C_5$, or hydroxy;

R_2 is indazol-5-yl, or indazol-6-yl;

n is an integer between 0 and 3.

BACKGROUND ART

Hepatitis B virus (HBV; referred as "HBV" hereinafter) causes acute or chronic hepatitis, which may progress to liver cirrhosis and liver cancer. It is estimated that three hundred

million people are infected with HBV in the world (Tiollais & Buendia, *Sci. Am.*, 264, 48, 1991). There has been much research about the molecular biological characteristics of HBV and their relationship to liver diseases in order to find ways to prevent and treat hepatitis B. Various vaccines and diagnostic drugs have been developed and much effort is being channeled into research to find treatment for hepatitis B.

HBV genome consists of genes for polymerase (P), surface protein (pre-S1, pre-S2 and S), core protein (pre-C and C), and X protein. Of these proteins expressed from HBV genes, polymerase, surface protein, and core protein are structural proteins and X protein has a regulatory function.

The gene for HBV polymerase comprises 80% of the whole virus genome and produces a protein of 94kD size with 845 amino acids, which has several functions in the replication of virus genome. This polypeptide includes sequences responsible for activities of protein primer, RNA dependent DNA polymerase, DNA dependent DNA polymerase, and RNase H. Kaplan and his coworkers first discovered reverse transcriptase activities of polymerase, which led to much research in replicating mechanism of HBV.

HBV enters liver when antigenic protein on virion surface is recognized by hepatic cell-specific receptor. Inside the liver cell, DNAs are synthesized by HBV polymerase action, attached to short chain to form complete double helix for HBV genome. Completed double helical DNA genome of HBV produces pre-genomic mRNA and mRNAs of core protein, surface protein, and regulatory protein by the action of RNA polymerase. Using these mRNAs, virus proteins are synthesized. Polymerase has an important function in the production of virus genome, forming a structure called replicasome with core protein and pre-genomic mRNA. This process is called encapsidation. Polymerase has repeated units of glutamic acid at the 3'-end with high affinity for nucleic acids, which is responsible for facile encapsidation. When replicasome is formed, (-) DNA strand is synthesized by reverse transcribing action of HBV polymerase and (+) DNA strand is made through the action of DNA dependent DNA polymerase, which in turn produces pre-genomic mRNAs. The whole process is repeated until the pool of more than 200 to 300 genomes is maintained (Tiollais and Buendia, *Scientific American*, 264: 48-54, 1991).

Although HBV and HIV are different viruses, the replication mechanisms during their proliferation have some common steps, namely, the reverse transcription of virus RNA to form DNA and the removal of RNA strand from subsequently formed RNA-DNA hybrid.

Recently, nucleoside compounds such as lamivudine and famvir have been reported to be useful inhibitors of HBV proliferation, although they have been originally developed as therapeutics for the treatment of acquired immune deficiency syndrome (AIDS; referred as "AIDS" hereinafter) and herpes zoster infection (Gerin, J. L, *Hepatology*, 14: 198-199, 1991; Lok, A. S. P., *J. Viral Hepatitis*, 1: 105-124, 1994; Dienstag, J. L. *et al.*, *New England Journal of Medicine*, 333: 1657-1661, 1995). However, these nucleoside compounds are considered a poor choice for treatment of hepatitis B because of their high cost and side effects such as toxicity, development of resistant virus and recurrence of the disease after stopping treatment. Effort to find therapeutics for hepatitis B among non-nucleoside compounds has been continued and antiviral effects against HBV have been reported for quinolone compounds (EPO 563732, EPO 563734), iridos compounds (KR 94-1886), and terephthalic amide derivatives (KR

96-72384, KR 97-36589, KR 99-5100). In spite of much effort, however, effective drugs for treating hepatitis B have not been developed yet and therapeutic method mainly depends on symptomatic treatment.

AIDS is a disease inducing dramatic decrease in immune function in the body cells and causing various symptoms of infection rarely seen in normal human beings, which spread to the whole body. Human immunodeficiency virus (HIV; referred as "HIV" hereinafter) responsible for AIDS is known to mainly attack helper T cells, which is one of the T cells with regulatory function in the immune system. When helper T cells are infected with HIV virus and undergo necrosis, human immune system cannot function properly. Impairment in immune function subsequently results in fatal infection and development of malignant tumor. Since AIDS patient has been found in USA in 1981 for the first time, the number increased to more than 850,000 patients in 187 countries in 1993 (WHO 1993 report). WHO predicted that 30 to 40 million more people would be infected with HIV by the year 2000 and 10 to 20 million of them would develop the disease.

At the present time, drugs controlling proliferation of HIV have been most widely used for the treatment of AIDS. Of these, Zidovudine, which had been named Azidothymidine previously, is a drug developed in 1987. Didanosine was developed in 1991 as an alternative medicine for AIDS patients when Zidovudine was either ineffective or could not be used due to side effects. In addition, Zalcitabine was approved for concurrent use with Zidovudine in 1992. These drugs alleviate symptoms, slow down progression of the disease in the infected individuals to full-blown AIDS, and somewhat extend life span in the patients. These drugs, however, are not able to cure the patients completely and often develop problems such as resistance and side effects.

In light of these problems, we, inventors of the present invention, tried to develop therapeutics to treat hepatitis B with little chance of toxicity, side effects, and development of resistant viral strains. We found non-nucleoside compounds with excellent antiviral effect against HBV; synthesized novel 3-nitropyridine derivatives represented in formula 1 and completed the invention by showing their dramatic inhibitory

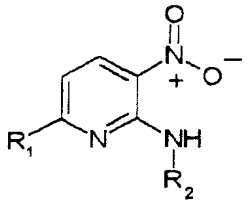
effect on proliferation of HIV as well as of HBV.

DISCLOSURE OF INVENTION

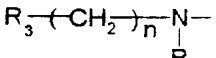
The present invention provides novel 3-nitropyridine derivatives and the pharmaceutical compositions containing said derivatives. More specifically, the present invention provides 3-nitropyridine derivatives and their pharmaceutically acceptable salts, the process for their preparation and the pharmaceutical compositions containing said derivatives as effective ingredient. 3-nitropyridine derivatives of the present invention inhibit proliferation of hepatitis B virus as well as of human immunodeficiency virus and may be effectively used for prevention and treatment of hepatitis B and AIDS.

In order to accomplish the aforementioned goal, the present invention provides novel 3-nitropyridine derivatives represented below in formula 1 and their pharmaceutically acceptable salts.

Formula 1



Wherein,

R_1 is methoxy or  ;

R_3 is H, hydroxy, dialkylamino group with $C_2 \sim C_6$, straight or branched hydroxyalkyl group with $C_2 \sim C_6$, straight or branched dihydroxyalkyl group with $C_3 \sim C_6$, alkoxyalkyl group with $C_3 \sim C_6$, or saturated or unsaturated 5 or 6 membered heterocyclic compounds containing 1 to 3 heteroatoms selected from N, O, and S, which may be unsubstituted or substituted with alkyl group with $C_1 \sim C_3$;

R_3 may or may not have asymmetrical carbons;

R_4 is H, straight or branched alkyl group with $C_1 \sim C_4$, or cycloalkyl group with $C_3 \sim C_6$;

R_3 and R_4 both may consist of 5 or 6 membered heterocyclic ring with 1 to 3 heteroatoms selected from N, O, and S, which is either unsubstituted or substituted with straight or branched alkyl group with $C_1 \sim C_5$, straight or branched hydroxyalkyl group with $C_2 \sim C_5$, or hydroxy;

R_2 is indazol-5-yl, or indazol-6-yl;

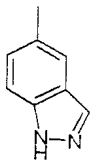
n is an integer between 0 and 3.

When both R_3 and R_4 are represented as a 5 or 6 membered heterocyclic compounds with 1 to 3 heteroatoms selected from N, O, and S, n equals 0. This heterocyclic ring may be unsubstituted or substituted with straight or branched alkyl group with $C_1 \sim C_5$, straight or branched hydroxyalkyl group with $C_2 \sim C_5$, or hydroxy group;

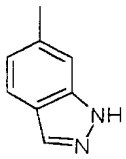
When compounds of formula 1 have asymmetrical carbons, they may exist as either *R* or *S* optical isomer and the present invention covers both optical isomers and the racemic mixture as well.

Indazol-5-yl and indazol-6-yl groups for R_2 in the present invention are represented in formula 2 and 3 respectively.

Formula 2



Formula 3

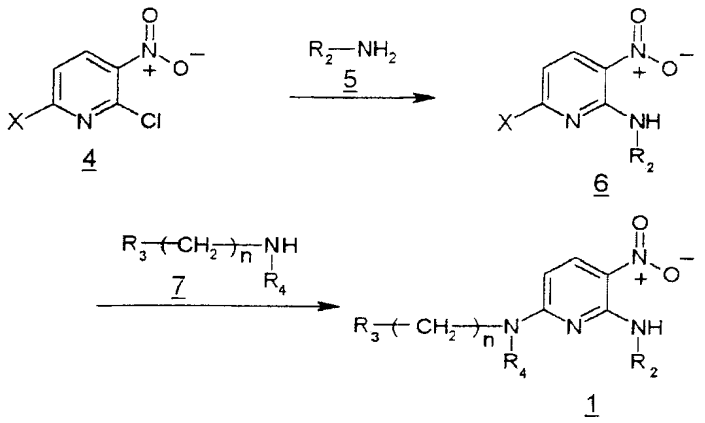


Compounds of formula 1 in the present invention may be utilized in the form of salts and the acid addition salts prepared by adding pharmaceutically acceptable free acids are useful. Compounds of formula 1 may be changed to the corresponding acid addition salts according to the general practices in this field. Both inorganic and organic acids may be used as free acids in this case. Among inorganic acids, hydrochloric acid, hydrobromic acid, sulfuric acid, and phosphoric acid may be used. Among organic acids, citric acid, acetic acid, lactic acid, tartaric acid, maleic acid, fumaric acid, formic acid, propionic acid, oxalic acid, trifluoroacetic acid, benzoic acid, gluconic acid, methanesulfonic acid, glycolic acid, succinic acid, 4-toluenesulfonic acid, galacturonic acid, embonic acid, glutamic acid and aspartic acid may be used.

The present invention also provides the process for preparing 3-nitropyridine derivatives of formula 1 as

represented in scheme 1.

Scheme 1



Wherein, X is Cl or OCH_3; R_2, R_3, R_4 and n are as defined in formula 1.

The process of preparation in the present invention comprises the following steps:

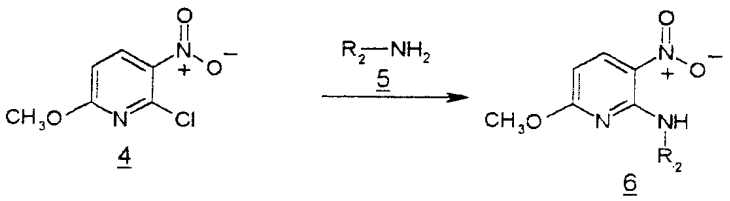
(Step 1) Synthesis of 3-nitropyridine derivatives of formula 6 by reacting 2-chloro-3-nitropyridine derivatives of formula 4 with 5-aminoindazole or 6-aminoindazole of formula 5 in a proper solvent under a basic condition at an appropriate temperature;

(Step 2) Synthesis of 3-nitropyridine derivatives of formula 1 by reacting 3-nitropyridine derivatives of formula

6 prepared in step 1 with appropriate amine compounds of formula 7 in a proper solvent under a basic condition at an appropriate temperature.

When R_1 in the compound of formula 1 is a methoxy group, step 1 completes the synthesis of desired compound ($X=OCH_3$). In this case, the present invention includes the method of preparing 6-methoxy-3-nitropyridine derivatives of formula 6 by reacting 2-chloro-6-methoxy-3-nitropyridine of formula 4 with 5-aminoindazole or 6-aminoindazole of formula 5 in the presence of a base.

Scheme 2



Compound of formula 4 in the first step of scheme 1 is 2-chloro-6-methoxy-3-nitropyridine or 2,6-dichloro-3-nitropyridine.

Chemical reagents used in the first and the second steps of scheme 1, namely, 2-chloro-3-nitropyridine derivatives of

formula 4, 5-aminoindazole or 6-aminoindazole of formula 5, and amine compounds of formula 7, are commercially available and may be purchased.

Compound of formula 7 in the step 2 above is used to introduce a substituent (R_3-$(CH_2)_n$-NR_4-) into the compound of formula 1 and an appropriate amine compound should be selected depending on the substituent desired, which can be easily done by one with general knowledge in the technical field.

To give more specific details about step 1 in the synthetic process, an organic base may be used and common tertiary amines such as triethylamine, *N,N*-diisopropylethylamine, *N*-methylmorpholine, *N*-methylpiperidine, 4-dimethylaminopyridine, *N,N*-dimethylaniline, 2,6-lutidine, pyridine are preferable.

Preferable reaction time and temperature are 4～15 hrs and 20～60 ℃.

Preferable for the reaction is a single or a mixture of solvents selected from chloroform, methylene chloride, acetonitrile and alcohols such as methanol and ethanol.

Of 3-nitropyridine derivatives of formula 6 produced in the reaction of step 1, one with chloro group at 6 position is

used in the following reaction of step 2.

The reaction in step 2 is described in more detail. Preferable solvent is a single or a mixture of solvents selected from acetonitrile, chloroform, methylene chloride, tetrahydrofuran, *N,N*-dimethylformamide, *N*-methylpyrrolidinone, pyridine, water and alcoholic solvents such as methanol, ethanol, and isopropanol.

It is preferable to use excess amount of amine compound (formula 7) to increase the efficiency of the reaction. Solvents used in the previous step 1 for the synthesis of 3-nitropyridine derivatives of formula 6 are preferable. Reaction temperature of 25~80 ℃ is preferable although it depends on the kind of amine compound used.

In another aspect of this invention, also provided are the pharmaceutical compositions of therapeutics for preventing and treating hepatitis B, which contain 3-nitropyridine derivatives of formula 1 and their pharmaceutically acceptable salts as effective ingredients.

The present invention also provides the pharmaceutical

compositions of therapeutics for preventing and treating AIDS, which contain 3-nitropyridine derivatives and their pharmaceutically acceptable salts of formula 1 as effective ingredients.

3-nitropyridine derivatives of formula 1 in this invention have inhibitory effect on proliferation of both HIV and HBV because they interfere with removal of RNA strand from RNA-DNA hybrid formed during the reverse transcription of viral RNA to DNA, which is a common step in the replication mechanism of the two viruses.

Compounds of formula 1 may be taken orally as well as through other routes in clinical uses; for example, it may be administered intravenously, subcutaneously, intraperitoneally, or locally and used in the form of general drugs.

For clinical use of drugs with the pharmaceutical compositions of the present invention, compounds of formula 1 may be mixed with pharmaceutically acceptable excipients and made into various pharmaceutically acceptable forms; for example, tablets, capsules, trochese, solutions, suspensions for oral administration; and injection solutions, suspensions, or dried

powder to be mixed with distilled water for the formulation of instant injection solution.

Effective dosage for compound of formula 1 is generally 10~500 mg/kg, preferably 50~300 mg/kg for adults, which may be divided into several doses, preferably into 1~6 doses per day if deemed appropriate by a doctor or a pharmacist.

BEST MODE FOR CARRYING OUT THE INVENTION

Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

<Preparation Example 1> Preparation of 6-chloro-2-(1*H*-5-indazolylamino)-3-nitropyridine.

To the solution of 2,6-dichloro-3-nitropyridine (4 g) and 5-aminoindazole (2.8 g) in acetonitrile (50 ml) was added triethylamine (3.2 ml), and then the solution was reacted at

20-25℃ for 12 hr. The reaction mixture was cooled at room temperature, added H_2O 30 ml slowly, and then reacted at 20℃ for 1 hr. The reaction mixture was filtered, washed with the mixture solution(15 ml) of acetonitrile : H_2O = 1: 1 (volume ratio) and dried at 50℃ *in vacuo* to obtain the desired compound (4.7 g, 78%).

m.p. : 233 ℃ (dec.)

^{1}H-NMR (DMSO-d_6), ppm : δ 6.94(d, 1H), 7.44(d, 1H), 7.56(d, 1H), 7.91(s, 1H), 8.08(s, 1H), 8.53(d, 1H), 10.20(s, 1H), 13.12(br s, 1H)

<Preparation Example 2> Preparation of 6-chloro-2-(1*H*-6-indazolylamino)-3-nitropyridine.

To the solution of 2,6-dichloro-3-nitropyridine (4 g) and 6-aminoindazole (2.8 g) in acetonitrile (50 ml) was added triethylamine (3.2 ml), and then the solution was reacted at 35-40℃ for 12 hr. The reaction mixture was cooled at room temperature with slowly adding H_2O (20 ml), and then reacted at 20-25℃ for 1 hr. The reaction mixture was filtered, washed with acetonitrile (6 ml) and H_2O (15 ml), and then dried at 50℃ *in vacuo* to obtain the desired compound (4.4 g, 73%).

m.p. : 234 ℃ (dec.)

^{1}H-NMR (DMSO-d_6), ppm : δ 7.02(d, 1H), 7.20(d, 1H), 7.73(d, 1H), 7.99(d, 2H), 8.55(d, 1H), 10.26(s, 1H), 13.09(s, 1H)

<Example 1> Preparation of 2-(1*H*-5-indazolylamino)-6-methoxy-3-nitropyridine.

To the solution of 2-chloro-6-methoxy-3-nitropyridine (5 g) and 5-aminoindazole (3.7 g) in methanol (60 ml) was added triethylamine (4.1 ml), and then the solution was reacted at 25-30 ℃ for 5 hr. The reaction mixture was cooled at room temperature with slowly adding H_2O (30 ml), and then stirred for 0.5 hr. The reaction mixture was filtered, washed with methanol (10 ml), obtained a solid product. The solid product was dried at 50℃ *in vacuo* to obtain the desired compound (6.5 g, 86%).

m.p. : 206~208 ℃

^{1}H-NMR (DMSO-d^6), ppm : δ 3.80(s, 3H), 6.32(d, 1H), 7.55(m, 2H), 8.06(s, 2H), 8.43(d, 1H), 10.52(s, 1H), 13.09(br s, 1H)

<Example 2> Preparation of 2-(1*H*-6-indazolylamino)-6-methoxy-3-nitropyridine.

To the solution of 2-chloro-6-methoxy-3-nitropyridine (5 g) and 6-aminoindazole (3.9 g) in methanol (60 ml) was added triethylamine (4.1 ml), and then the solution was reacted at 55-60℃ for 14 hr. The reaction mixture was cooled at room temperature, added H_2O 30 ml slowly at 25℃, and then stirred for 0.5 hr. The reaction mixture was filtered, washed with 50% aqueous methanol solution (15 ml) and obtained a solid product. The solid product was dried at 50℃ *in vacuo* to obtain the desired compound (6.8 g, 90%).

m.p. : 261~264 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 3.94(s, 3H), 6.39(d, 1H), 7.24(m, 1H), 7.71(d, 1H), 8.01(s, 1H), 8.19(s, 1H), 8.44(d, 1H), 10.62(s, 1H), 13.04(br s, 1H)

<Example 3> Preparation of 2-(1*H*-5-indazolylamino)-6-methyl amino-3-nitropyridine.

To the solution of methanol with 40% methylamine (20 ml) was added 2-(1*H*-5-indazolylamino)-6-methoxy-3-nitropyridine (1 g) obtained by the example 1, and the solution was reacted at 25℃ for 1 hr. The reaction mixture was added H_2O (20ml) slowly and stirred for 1 hr. The reaction mixture was filtered, washed

with 30% aqueous methanol solution (5 ml) and then obtained a solid product. The solid product was dried at 50-60 ℃ *in vacuo* to obtain the desired compound (0.82 g, 82%).

m.p. : 238~240 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 2.86(d, 3H), 6.09(d, 1H), 7.51(d, 1H), 7.57(d, 1H), 8.05(t, 2H), 8.24(d, 2H), 10.97(s, 1H), 13.05(br s, 1H)

<Example 4> Preparation of 2-(1*H*-6-indazolylamino)-6-methylamino-3-nitropyridine.

To the solution of methanol with 40% methylamine (20 ml) was added 2-(1*H*-6-indazolylamino)-6-methoxy-3-nitropyridine (1 g) obtained by the example 2, and the solution was reacted at 25-30℃ for 2 hr. The reaction mixture was cooled and stirred at 20℃ for 0.5 hr. The reaction mixture was filtered, washed with methanol (4 ml) and then obtained a solid product. The solid product was dried at 40℃ *in vacuo* to obtain the desired compound (0.79 g, 79%).

m.p. : > 270 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 2.98(d, 3H), 6.15(d, 1H), 7.18(d, 1H), 7.69(d, 1H), 7.99(s, 1H), 8.09(d, 1H), 8.35(br s, 1H), 8.44(s,

1H), 11.14(s, 1H), 13.03(br s, 1H)

<Example 5> Preparation of 2-(1*H*-5-indazolylamino)-6-isopropyl amino-3-nitropyridine.

To the solution of 2-(1*H*-5-indazolylamino)-6-methoxy-3-nitropyridine (1 g) obtained by the example 1 in methanol (20 ml) was added isopropylamine (20 ml) slowly and reacted at 45℃ for 20 hr. The reaction mixture was cooled, added H_2O (60 ml) at 25℃ and then stirred for 1 hr. The reaction mixture was filtered, washed with 20 % aqueous methanol solution (5 ml) and then obtained a solid product. The solid product was dried at 50~60℃ *in vacuo* to obtain the desired compound (1.05 g, 96%).

m.p. : 233~235 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 1.15(d, 6H), 4.03(m, 1H), 6.06(d, 1H), 7.50(d, 2H), 8.05(m, 2H), 8.15(t, 2H), 10.97(s, 1H), 13.06(br s, 1H)

<Example 6> Preparation of 2-(1*H*-6-indazolylamino)-6-isopropyl amino-3-nitropyridine.

To the solution of

2-(1*H*-6-indazolylamino)-6-methoxy-3-nitropyridine (1 g) obtained by the example 2 in methanol (20 ml) was added isopropylamine (20 ml) slowly and reacted at 45℃ for 45 hr. The reaction mixture was cooled and stirred at 25℃ for 1 hr. The reaction mixture was filtered, washed with methanol (5 ml) and then obtained a solid product. The solid product was dried at 40~50℃ *in vacuo* to obtain the desired compound (0.95 g, 87%).

m.p. : > 270℃

^{1}H-NMR (DMSO-d_6), ppm : δ 1.23(d, 6H), 4.17(m, 1H), 6.12(d, 1H), 7.15(d, 1H), 7.68(d, 1H), 8.00(s, 1H), 8.09(d, 1H), 8.28(d, 1H), 8.35(s, 1H), 11.12(s, 1H), 13.08(br s, 1H)

<Example 7> Preparation of 2-(1*H*-5-indazolylamino)-6-isobuthylamino-3-nitropyridine.

To the solution of 2-(1*H*-5-indazolylamino)-6-methoxy-3-nitropyridine (1 g) obtained by the example 1 in methanol (20 ml) was added isobuthylamine (15 ml) slowly and reacted at 45~50℃ for 20 hr. The reaction mixture was cooled, added H_2O (40 ml) slowly and then stirred at 25℃ for 1 hr. The reaction mixture was filtered, washed with 30% aqueous methanol solution (5 ml), obtained a

solid product. The solid product was dried at 50~60℃ *in vacuo* to obtain the desired compound (0.95 g, 83%).

m.p. : 230~232 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 0.83(d, 6H), 1.83(m, 1H), 3.11(d, 2H), 6.11(d, 1H), 7.50(s, 2H), 7.99(s, 1H), 8.06(d, 1H), 8.19(d, 1H), 8.39(t, 1H), 10.91(s, 1H), 13.07(br s, 1H)

<Example 8> Preparation of 6-cyclopropylamino-2-(1*H*-5-indazolylamino)-3-nitropyridine.

To the solution of 2-(1*H*-5-indazolylamino)-6-methoxy-3-nitropyridine (1 g) obtained by the example 1 in methanol (20 ml) was added cyclopropylamine (10 ml) slowly, heated and reacted at 40~45℃ for 25 hr. The reaction mixture was cooled with adding H_2O (40 ml) slowly and stirred at 25℃ for 1 hr. The reaction mixture was filtered, washed with 30% aqueous methanol solution (5 ml), obtained a solid product. The solid product was dried at 50℃ *in vacuo* to obtain the desired compound (0.82 g, 75%).

m.p. : 237~240 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 0.56(m, 2H), 0.81(m, 2H), 2.81(br s,1H), 6.06(d, 1H), 7.50(d, 1H), 7.62(d, 1H), 8.02(s, 1H), 8.09(d,

1H), 8.46(s, 1H), 8.57(s, 1H), 11.02(s, 1H), 13.04(br s, 1H)

<Example 9> Preparation of 6-amino-2-(1*H*-5-indazolylamino)-3-nitropyridine.

To the solution of 6-chloro-2-(1*H*-5-indazolylamino)-3-nitropyridine (1 g) obtained by the preparation example 1 in chloroform (20 ml) was added 7 N ammonia solution in methanol (30 ml) and reacted at 35~40 ℃ for 15 hr. The reaction mixture was cooled, concentrated under reduced pressure at 25 ℃ and then precipitated with treatment of methanol (10 ml). The reaction mixture was filtered, which was recrystallised with methanol : methylene chloride = 4: 1 to obtain the desired compound (0.63 g, 67%)

m.p. : 263 ℃ (dec.)

^{1}H-NMR (DMSO-d_6), ppm : δ 6.05(d, 1H), 7.48(m, 2H), 7.56(br s, 1H), 7.65(br s, 1H), 8.01(s, 1H), 8.12(d, 1H), 8.28(s, 1H), 10.81(s, 1H), 13.06(br s, 1H)

<Example 10> Preparation of 6-(2-hydroxyethyl)methylamino-2-(1*H*-5-indazolylamino)-3-nitropyridine.

To the solution of 6-chloro-2-(1*H*-5-indazolylamino)-3-nitropyridine (1 g) obtained by the preparation example 1 in acetonitrile (20 ml) was added 2-(methylamino)ethanol (1.4 ml) and triethylamine (0.6 ml) and then refluxed for 12 hr. The reaction mixture was cooled, precipitated with adding excess H_2O at 20~25℃, filtered to obtain solid. The above obtained solid was washed with water, dried at 50℃ *in vacuo* and recrystallised with chloroform : ether = 1: 3 to obtain the desired compound (0.7 g, 62%).

m.p. : 172~174 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 3.14(s, 3H), 3.64(m, 4H), 4.80(d, 1H), 6.36(d, 1H), 7.50(s, 2H), 8.02(s, 1H), 8.15(m, 2H), 10.71(d, 1H), 13.03(br s, 1H)

It was prepared compounds in prepared example 11~30 as the same method used for the example 10. It is shown in Table 1 that the compound name, yield, recrystallizing solution, melting point of compounds in prepared example 11~30 and 3-nitropyridine derivatives (6) and amine compound (7) as starting materials. It is shown in Table 2 that ^{1H}-NMR of compounds in prepared example 11~30.

TABLE 1a

Example	Compounds				
	3-nitropyridine derivatives (6)	amine compound (7)	Recrystallising solv.	Yield (%)	m.p. (℃)
11	6-ethyl-(2-hydroxyethyl)amino-2-(1*H*-5-indazolylamino)-3-nitropyridine				
	Preparation example 1	2-(ethylamino)ethanol	methanol/ether (1:2)	52	164~166
12	6-[(1*S*)-1-(hydroxyethyl)ethylamino]-2-(1*H*-5-indazolylamino)-3-nitropyridine				
	Preparation example 1	(*S*)-2-amino-1-propanol	ethanol	75	>270
13	6-[(1*S*)-1-(hydroxyethyl)ethylamino]-2-(1*H*-6-indazolylamino)-3-nitropyridine				
	Preparation example 2	(*S*)-2-amino-1-propanol	ethanol	69	264~265
14	6-[bis(hydroxymethyl)methylamino]-2-(1*H*-5-indazolylamino)-3-nitropyridine				
	Preparation example 1	2-amino-1,3-propanediol	methanol	80	>270
15	2-(1*H*-5-indazolylamino)-6-(2-methoxy-1-methyl)ethylamino-3-nitropyridine				
	Preparation example 1	2-amino-1-methoxypropane	chloroform/ether (1:5)	58	138~142
16	6-[2-(dimethylamino)ethylamino]-2-(1*H*-6-indazolylamino)-3-nitropyridine				
	Preparation example 2	*N,N*-dimethylethylenediamine	methanol/H_2O (1:2)	84	219~221
17	2-(1*H*-5-indazolylamino)-6-(4-methyl-1-piperazinyl)amino-3-nitropyridine				
	Preparation example 1	1-amino-4-methylpiperazine	mehanol/ether (1:4)	78	170~174
18	2-(1*H*-6-indazolylamino)-6-(4-methyl-1-piperazinyl)amino-3-nitropyridine				
	Preparation example 2	1-amino-4-methylpiperazine	methanol	56	260 (dec.)
19	2-(1*H*-5-indazolylamino)-3-nitro-6-(3-pyridyl)methylaminopyridine				
	Preparation example 1	3-(aminomethyl)pyridine	methanol/H_2O (1:1)	81	250~253
20	2-(1H-6-indazolylamino)-3-nitro-6-(3-pyridyl)methylaminopyridine				
	Preparation example 2	3-(aminomethyl)pyridine	ethanol	77	>270
21	2-(1*H*-5-indazolylamino)-3-nitro-6-(4-pyridyl)methylaminopyridine				
	Preparation example 1	4-(aminomethyl)pyridine	ethanol/H_2O (2:1)	75	219~221
22	2-(1*H*-6-indazolylamino)-3-nitro-6-(2-pyridyl)methylaminopyridine				
	Preparation example 2	2-(aminomethyl)pyridine	methanol/ethanol (1:1)	72	256 (dec.)
23	2-(1*H*-5-indazolylamino)-3-nitro-6-(1-piperazinyl)pyridine				
	Preparation example 1	piperazine	ethanol	78	270 (dec.)

TABLE 1b

Example	Compounds				
	3-nitropyridine derivatives (6)	amine compound (7)	Recrystallising soln	Yield (%)	m.p. (℃)
24	2-(1*H*-6-indazolylamino)-3-nitro-6-(1-piperazinyl)pyridine				
	Preparation example 2	piperazine	ethanol	88	268 (dec.)
25	2-(1*H*-5-indazolylamino)-6-(4-methyl-1-piperazinyl)-3-nitropyridine				
	Preparation example 1	1-methylpiperazine	methylene chloride/isopropyl ether (1:5)	63	116~120
26	2-(1*H*-6-indazolylamino)-6-(4-methyl-1-piperazinyl)-3-nitropyridine				
	Preparation example 2	1-methylpiperazine	ethanol/H_2O (1:1)	60	253 (dec.)
27	6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-(1*H*-6-indazolylamino)-3-nitropyridine				
	Preparation example 2	N-(2-hydroxyethyl)piperazine	ethanol	82	215~217
28	6-(4-hydroxy-1-piperidinyl)-2-(1*H*-5-indazolylamino)-3-nitropyridine				
	Preparation example 1	4-hydroxypiperidine	ethanol	76	235~239
29	6-(4-hydroxy-1-piperidinyl)-2-(1*H*-6-indazolylamino)-3-nitropyridine				
	Preparation example 2	4-hydroxypiperidine	methanol	80	270~272
30	2-(1*H*-5-indazolylamino)-6-(4-morpholinyl)amino-3-nitropyridine				
	Preparation example 1	4-aminomorpholine	methanol	64	>270

TABLE 2a

Example	NMR solution	^{1}H-NMR data (ppm)
11	DMSO-d_6	δ1.09(d, 3H), 3.56(s, 6H), 4.80(d, 1H), 6.35(d, 1H),7.49(s, 2H), 8.01(s, 1H), 8.15(m, 2H), 10.68(s, 1H), 13.03(br s, 1H)
12	DMSO-d_6	δ1.13(d, 3H), 3.43(t, 2H), 4.01(br s, 1H), 4.81(t, 1H), 6.14(d, 1H), 7.51(t, 2H), 8.01(s, 1H), 8.06(d, 1H), 8.13(d, 1H), 8.20(s, 1H), 10.92(s, 1H), 13.03(br s, 1H)
13	DMSO-d_6	δ1.18(d, 3H), 3.51(m, 2H), 4.13(br s, 1H), 4.81(d, 1H), 6.19(d, 1H), 7.20(d, 1H), 7.69(d, 1H), 8.00(s, 1H), 8.08(d, 1H), 8.25(d, 2H), 11.09(br s, 1H), 13.03(br s, 1H)
14	DMSO-d_6	δ3.54(s, 4H), 4.00(s, 1H), 4.76(s, 2H), 6.21(d, 1H), 7.51(m, 2H), 8.00(s, 1H), 8.06(d, 1H), 8.12(d, 1H), 8.23(s, 1H), 10.93(s, 1H), 13.02(br s, 1H)
15	DMSO-d_6	δ1.12(d, 3H), 3.19(s, 3H), 3.26(m, 1H), 3.37(m, 1H), 4.13(br s, 1H), 6.11(d, 1H), 7.50(s, 2H), 8.00(s, 1H), 8.07 (d, 1H), 8.16(t, 2H), 10.86(s, 1H), 13.06(br s, 1H)
16	DMSO-d_6	δ2.11(s, 6H), 2.45(s, 2H), 3.49(s, 2H), 6.21(d, 1H), 7.23(d, 1H), 7.69(d, 1H), 8.00(s, 1H), 8.09(d, 1H), 8.17(s, 1H), 8.28(br s 1H), 11.04(s, 1H), 13.01(br s, 1H)
17	DMSO-d_6	δ2.30(s, 3H), 2.83(br s, 6H), 3.16(s, 2H), 6.49(d, 1H), 7.50(t, 2H), 8.03(s, 1H), 8.25(t, 2H), 9.15(s, 1H), 10.69(s, 1H), 13.06(s, 1H)
18	DMSO-d_6 +TFA-d_1	δ2.80(s, 3H), 2.99(br s,2H), 3.06(br s, 2H), 3.23(br s, 2H), 3.46(br s, 2H), 6.54(br s, 1H), 7.27(d, 1H), 7.71(d, 1H), 8.14(s, 1H), 8.26(br s, 2H)
19	DMSO-d_6	δ4.51(s, 2H), 6.18(m, 1H), 7.26(br s, 1H), 7.38(d, 1H), 7.43(d, 1H), 7.51(d, 1H), 7.93(s, 2H), 8.14(d, 1H), 8.42(s, 2H), 8.77(br s, 1H), 10.80(s, 1H), 13.03(br s, 1H)
20	DMSO-d_6	δ4.62(s, 2H), 6.22(d, 1H), 7.15(d, 1H), 7.27(br s, 1H), 7.64(d, 2H), 8.00(s, 1H), 8.15(s, 2H), 8.42(s, 1H), 8.50(s, 1H), 8.80(br s, 1H), 10.99(s, 1H), 13.01(br s, 1H)
21	DMSO-d_6	δ4.51(s, 2H), 6.23(d, 1H), 7.15(s, 2H), 7.32(m, 2H), 7.85(d, 2H), 8.16(d, 1H), 8.43(m, 2H), 8.79(t, 1H), 10.77(s, 1H), 13.01(br s, 1H)
22	DMSO-d_6	δ4.70(s, 2H), 6.30(d, 1H), 7.15(d, 1H), 7.25(m, 2H), 7.58(d, 1H), 7.66(t, 1H), 7.99(s, 1H), 8.06(s, 1H), 8.15(d, 1H), 8.52(d, 1H), 8.85(br s, 1H), 10.98(s, 1H) 12.98(br s, 1H)
DMSO :dimethylsulfoxide, TFA : trifluoroacetic acid		

TABLE 2b

Example	NMR solution	^{1}H-NMR data (ppm)
23	DMSO-d_6	δ2.70(s, 4H), 3.59(s, 4H), 6.46(d, 1H), 7.51(s, 2H), 7.96(s, 1H), 8.03(s, 1H), 8.16(d, 1H), 10.55(s, 1H), 13.07(br s, 1H)
24	DMSO-d_6	δ2.76(s, 4H), 3.66(s, 4H), 6.51(d, 1H), 7.16(d, 1H), 7.69(d, 1H), 8.00(s, 1H), 8.07(d, 1H), 8.20(d, 1H), 10.82(s, 1H), 13.07(br s, 1H)
25	DMSO-d_6	δ2.17(s, 3H), 2.34(s, 4H), 3.65(s, 4H), 6.48(d, 1H), 7.51(t, 2H), 7.96(d, 1H), 8.04(d, 1H), 8.18(d, 1H), 10.59(s, 1H), 13.07(br s, 1H)
26	DMSO-d_6	δ2.21(s, 3H), 2.40(s, 4H), 3.74(br s, 4H), 6.54(d, 1H), 7.17(d, 1H), 7.70(d, 1H), 8.00(s, 1H), 8.07(d, 1H), 8.22(d, 1H), 10.78(s, 1H), 13.06(br s, 1H)
27	DMSO-d_6+TFA-d_1	δ3.19(t, 4H), 3.59(m, 4H), 3.74(t, 2H), 4.51(br s, 2H), 6.50(d, 1H), 7.17(d, 1H), 7.69(d, 1H), 8.08(d, 2H), 8.28(d, 1H)
28	DMSO-d_6	δ1.35(m, 2H), 1.76(d, 2H), 3.37(d, 2H), 3.75(br s, 1H), 3.99(br s, 2H), 4.78(s, 1H), 6.50(d, 1H), 7.51(s, 2H), 7.98(d, 1H), 8.02(s, 1H), 8.17(d, 1H), 10.61(s, 1H), 13.05(br s, 1H)
29	DMSO-d_6	δ1.42(m, 2H), 1.82(d, 2H), 3.45(t, 2H), 3.79(br s, 1H), 4.06(br s, 2H), 4.82(s, 1H), 6.55(d, 1H), 7.18(d, 1H), 7.69(d, 1H), 8.00(s, 1H), 8.05(s, 1H), 8.19(d, 1H), 10.81(s, 1H), 13.06(s, 1H)
30	DMSO-d_6	δ2.77(s, 4H), 3.68(s, 4H), 6.55(s, 1H), 7.50(t, 2H), 8.02(s, 1H), 8.25(t, 2H), 9.12(s, 1H), 10.67(s, 1H), 13.04(s, 1H)
DMSO :dimethylsulfoxide, TFA : trifluoroacetic acid		

<Experiment 1> Inhibitory effect on the in vitro activities of HBV polymerase in reverse transcription

The following in vitro experiment was performed to determine the effect of the compounds of formula 1 on the activity of HBV

polymerase during reverse transcription.

The present inventors submitted application for a patent concerning HBV polymerase genetically expressed in and separated from *E.coli*, the process of its preparation, and the method to measure the enzyme activities (KR 94-3918, KR 96-33998). In the present experiments HBV polymerase was used which had been expressed in *E. coli* as stated above.

The method used in the present invention to measure in vitro reverse transcribing activities of HBV polymerase is as follows. Basic principles are the same as for ELISA. Nucleotides with biotin or digoxigenin group attached are included as substrates and anti-DIG antibodies attached to peroxidase enzyme recognize the polymerized substrates.

To the wells coated with streptavidin, 20㎕ of HBV polymerase, 20 ㎕ of reaction mixture (10 μM each of DIG-UTP and Biotin-UTP, 46 mM Tris-HCl, 266 mM KCl, 27.5 mM $MgCl_2$, 9.2 mM DTT substrate/primer hybrid), and 20 ㎕ of test compound(added to 1, 0.1, and 0.01 ㎍/㎖) were added and allowed to react at 22℃ for 15 hrs. During this reaction, HBV polymerase catalyzes DNA synthesis and digoxigenin and biotin attached to nucleotides form bonds with streptavidin coated on the bottom of wells. When

the reaction was done, each well was washed with 250 ㎕ of cleaning buffer (pH 7.0) for 30 seconds, which was repeated five times to remove remaining impurities. 200 ㎕ of anti-DIG-POD antibody was added to each well and allowed to react for 1 hr at 37℃, and the wells were washed with cleaning buffer to remove impurities. 200㎕ each of ABTS™, a substrate of peroxidase, was then added and allowed to react at room temperature for 30 min. Absorbance was measured at 405 nm using ELISA reader.

The percentage of reduction in HBV polymerase activities for reverse transcription was calculated using the group without test compound as a control and the results are shown in Table 3.

TABLE 3a Effect on the HBV polymerase activities in reverse transription

Compound	Inhibition activity on HBV-RT (%)		
	1 ㎍/㎖	0.1 ㎍/㎖	0.01 ㎍/㎖
Example 1	85	54	30
Example 2	76	50	12
Example 3	58	47	20
Example 4	60	51	26
Example 5	96	87	53
Example 6	91	76	49
Example 7	95	80	47
Example 8	72	52	38

TABLE 3b. Effect on the HBV polymerase activities in reverse transcription

Compound	Inhibition activity on HBV-RT (%)		
	1 μg/mℓ	0.1 μg/mℓ	0.01 μg/mℓ
Example 9	80	59	40
Example 10	78	58	50
Example 11	85	60	35
Example 12	90	56	38
Example 13	66	35	10
Example 14	85	49	30
Example 15	97	68	47
Example 16	80	55	38
Example 17	75	52	41
Example 18	70	43	25
Example 19	91	58	42
Example 20	66	40	21
Example 21	94	65	45
Example 22	81	60	49
Example 23	96	66	51
Example 24	62	43	20
Example 25	88	54	35
Example 26	63	40	18
Example 27	60	38	15
Example 28	55	34	20
Example 29	52	35	10
Example 30	71	49	32

As shown in Table 3, compounds of the present invention have excellent inhibitory effects on the HBV polymerase activities with more than 90% inhibition at the concentration of 1 μg/ml. Moreover, compounds of the present invention are not expected to have problems such as toxicity and development

of resistant viruses as observed in the use of nucleosides and may be applied together with nucleoside compounds due to different mechanisms of action.

In summary, compounds of the present invention effectively reduce the activities of HBV polymerase, inhibit replication and proliferation of HBV and may be useful as therapeutics for prevention and treatment of hepatitis B.

<Experiment 2> Inhibitory effect on the proliferation of HBV in HBV producing cell line

The following experiment was performed to determine inhibitory effects of compounds of formula 1 on the proliferation of HBV producing cell line.

To test for antiviral effect, replication and proliferation of HBV were measured in HepG 2.2.15, a human liver cancer cell line.

The cell concentration was adjusted to 1×10^5 cells/㎖ and 1 ㎖ was added to each well of a 24-well cell culture plate, which was then kept in a culture box for 3 - 4 days at 37℃ under 5% CO_2 until cells grew sufficiently, changing culture medium everyday. When the cells matured sufficiently, the test

compounds were added to the final concentrations of 0.01, 0.1, and 1 ㎍/㎖. One week after the addition of test compounds, the culture solution was centrifuged at 5,000 rpm for 10 min. 25 ㎕ of supernatant was transferred to a new tube and 5 ㎕ of lysis solution [0.54N NaOH, 0.06% NP40] was added to each tube. After keeping the tube at 37℃ for 1 hr, 30 ㎕ of neutralizing solution [0.09N HCl, 0.1M Tris-HCl, pH 7.4] was added as a reaction solution for competitive polymerase chain reaction (PCR).

PCR was performed using genetic sequence of HBV core protein as a matrix. PCR reaction was carried out by adding 1 unit of Taq polymerase enzyme to 25 pmol of each primer, 250 μM dNTP, 5 ㎕ of PCR reaction solution [0.54N NaOH, 0.06% NP40, 0.09N HCl, 0.1M Tris-HCl, pH 7.4].

DNA polymerized by PCR was electrophoresed on Agarose gel and quantitatively analyzed using an image analyzer (Gel Doc 1000, Bio-Rad) in order to evaluate the effect of compounds of the present invention on the reduction of HBV proliferation.

3TC (lamivudine) was used as a positive control at the same concentrations as those of the test compounds. The percentage of reduction in HBV proliferation was calculated using the group without test compound as a control and the results

are represented in Table 4.

TABLE 4 Inhibitory effect on the HBV proliferation

Compound	Inhibition activity on HBV-RT (%)		
	1 μg/mℓ	0.1 μg/mℓ	0.01 μg/mℓ
Example 1	82	45	20
Example 2	72	40	-
Example 3	53	35	-
Example 4	58	40	-
Example 5	94	81	41
Example 6	90	72	33
Example 7	93	75	30
Example 8	65	46	25
Example 11	83	45	20
Example 12	85	48	29
Example 14	80	41	22
Example 15	95	60	36
Example 19	85	51	30
Example 21	90	50	33
Example 23	92	55	35
Example 25	83	46	25
3TC	99	80	48

As shown above in Table 4, non-nucleoside compounds of the present invention have excellent inhibitory effect on the HBV polymerase activities in reverse transcription with more than 80% reduction of HBV proliferation at the concentration of 1 μg/mℓ. Moreover, compounds of the present invention, being non-nucleosides, may not have problems such as toxicity and early

development of resistant virus strains observed in the use of nucleoside substances. It is also expected that compounds of the present invention may be used in parallel with nucleoside compounds since the former act on allosteric binding pockets while the latter act in the domain of polymerase activities.

As described above, compounds of the present invention have excellent inhibitory effect on the HBV polymerase activities important in reverse transcription step of HBV replication. Based on the mechanism, these compounds are able to effectively control HBV proliferation and may be useful as therapeutics for prevention and treatment of hepatitis B.

<Experiment 3> Inhibitory effect on the in vitro HIV enzyme activities in reverse transcription

The following in vitro experiments were done to determine the effect of compounds of formula 1 on the reduction of HIV enzyme activities in reverse transcription.

Non-radioactive reverse transcriptase assay kit (Boehringer Mannheim) was used in the measurement of in vitro transcriptase activities. 20 ㎕ (40 ng) of HIV transcriptase and 20 ㎕ of reaction mixture containing matrix-primer hybrid

poly(A)oligo(dT)$_{15}$, DIG(digoxigenin)-dUTP, biotin-dUTP, and TTP were added to wells coated with streptavidin. Test compounds were also added at the final concentrations of 0.1 and 1 μg/mℓ and allowed to react at 37℃ for 1 hr. At this time, DNA is formed from RNA by the action of HIV reverse transcriptase, forming bonds with streptavidin coated on the bottom of wells because of digoxigenin and biotin moieties attached to nucleotides.

When the reaction was completed, each well was washed with 250 μℓ of cleaning buffer (pH 7.0) for 30 sec. five times to remove remaining impurities. 200 μℓ of anti-DIG-POD antigen was added to each well, allowed to react at 37℃ for 1 hr an washed as above to remove impurities. 200 μℓ of ABTS™, a substrate for peroxidase, was added to each well and allowed to react at room temperature for 30min. Absorbance at 405 nm was then read for each solution using ELISA reader and used for quantitative determination of inhibitory effect on the HIV transcriptase activities. The percentage of reduction in the activities of HIV reverse transcriptase was calculated using the group without test compound as control and the results are represented in Table 5.

TABLE 5 Inhibitory effect on the activities of HIV reverse transcriptase

Compound	Inhibition activity on HBV-RT (%)	
	1 μg/mℓ	0.1 μg/mℓ
Example 1	75	35
Example 4	70	51
Example 5	55	20
Example 6	69	50
Example 7	72	45
Example 11	67	46
Example 12	64	40
Example 14	59	44
Example 16	75	51
Example 20	84	53
Example 21	72	39
Example 27	81	45

As shown above in Table 5, compounds of the present invention have excellent inhibitory effect on the activities of HIV reverse transcriptase, having more than 70% reduction at the concentration of 1 μg/mℓ. Moreover, it is expected that compounds of the present invention, being non-nucleosidic, do not have problems such as toxicity and early development of resistant virus strains observed in the use of nucleoside substances. Furthermore, compounds of the present invention may be used together with nucleoside compounds since the former act on allosteric binding pockets while the latter act in the domain

of polymerase activities.

As described above, compounds of the present invention have excellent inhibitory effect on the HIV enzyme activities in reverse transcription, which is a step in HIV replication. Based on the mechanism, these compounds are able to effectively control HIV proliferation and may be useful as therapeutics for prevention and treatment of AIDS.

<Experiment 4> Cytotoxicity test

To determine if compounds of formula 1 exhibit cytotoxicity, in vitro tests were carried out using HepG2 cells with MTT analysis method as generally known and the results are in Table 6 shown below.

TABLE 6 Cytotoxicity tests on HepG2 cells

Compound	Cytotoxicity on HepG2	
	IC_{50} [1)]	MCD [2)]
Example 2	>100	100
Example 6	>100	100
Example 11	>100	100

1) IC_{50}: 50% Inhibitory Concentration (㎍/㎖)

2) MCD: Minimal Cytotoxic Concentration (㎍/㎖)

As shown above in Table 6, compounds used in the experiments

have higher than 100 μg/mℓ for IC_{50} and are considered to have little cytotoxicity.

<Experiment 5> Acute toxicity in rats tested via oral administration

The following experiments were performed to see if compounds of formula 1 have acute toxicity in rats.

6-week old SPF SD line rats were used in the tests for acute toxicity. Compounds in the examples of 1~22 were suspended in 0.5% methylcellulose solution and orally administered once to 6 rats per group at the dosage of 2 g/kg/15mℓ. Death, clinical symptoms, and weight change in rats were observed, hematological tests and biochemical tests of blood performed, and any abnormal signs in the gastrointestinal organs of chest and abdomen checked with eyes during autopsy. The results showed that the test compounds did not cause any specific clinical symptoms, weight change, or death in rats. No change was observed in hematological tests, biochemical tests of blood, and autopsy. The compounds used in this experiment are evaluated to be safe substances since they do not cause any toxic change in rats up to the level of 2 g/kg and their estimated LD_{50} values are much

greater than 2 g/kg in rats.

INDUSTRIAL APPLICABILITY

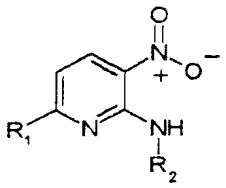
As described above, novel 3-nitropyridine derivatives of formula 1 in the present invention have dramatic inhibitory effect on proliferation of HBV and of HIV with little side effect and may be useful as therapeutic agents for prevention and treatment of hepatitis B and AIDS.

Moreover, it is expected that compounds of the present invention, being non-nucleosidic, do not have problems such as toxicity and early development of resistant virus strains observed in the use of nucleoside substances. Furthermore, compounds of the present invention may be used together with nucleoside compounds since the former seem to act on allosteric binding pockets while the latter work in the domain of polymerase activities.

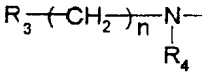
WHAT IS CLAIMED IS;

1. 3-Nitropyridine derivatives and their pharmaceutically acceptable salts as represented by formula 1.

Formula 1



Wherein,

R_1 is methoxy or  ;

R_3 is H, hydroxy, dialkylamino group with $C_2 \sim C_6$, straight or branched hydroxyalkyl group with $C_2 \sim C_6$, straight or branched dihydroxyalkyl group with $C_3 \sim C_6$, alkoxyalkyl group with $C_3 \sim C_6$, or saturated or unsaturated heterocyclic compounds containing 1 to 3 heteroatoms selected from N, O, and S, which may be unsubstituted or substituted with alkyl group of $C_1 \sim C_3$; R_3 may or may not contain asymmetrical carbons;

R_4 is H, straight or branched alkyl group with $C_1 \sim C_4$, or cycloalkyl group with $C_3 \sim C_6$;

R_3 and R_4 both may consist of 5 or 6 membered heterocyclic ring containing $1 \sim 3$ heteroatoms selected from N, O, and S, which

is unsubstituted or substituted with straight or branched alkyl group with $C_1 \sim C_5$, straight or branched hydroxyalkyl group with $C_2 \sim C_5$, or hydroxy;

R_2 is indazol-5-yl, or indazol-6-yl;

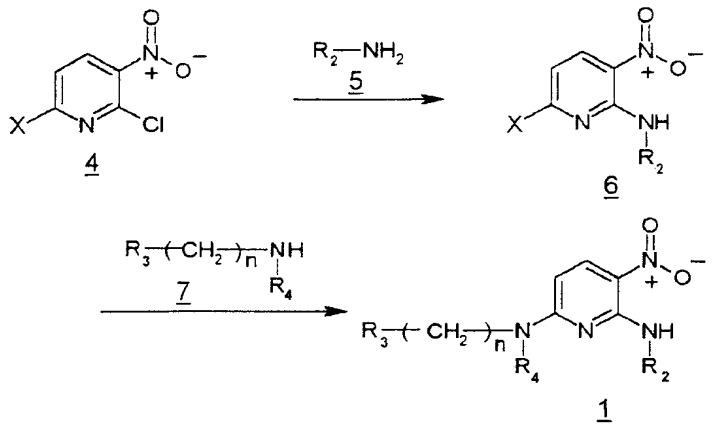
n is an integer between 0 and 3.

2. Process for the preparation of 3-nitropyridine derivatives of formula 1 comprising the following two steps as represented in scheme 1:

Step 1. Synthesis of 3-nitropyridine derivatives of formula 6 by reacting 2-chloro-3-nitropyridine derivatives of formula 4 with 5-aminoindazole or 6-aminoindazole of formula 5 in the presence of a base;

Step 2. Preparation of 3-nitropyridine derivatives of formula 1 by reacting 3-nitropyridine derivatives of formula 6 synthesized in step 1 with amine compounds of formula 7

Scheme 1

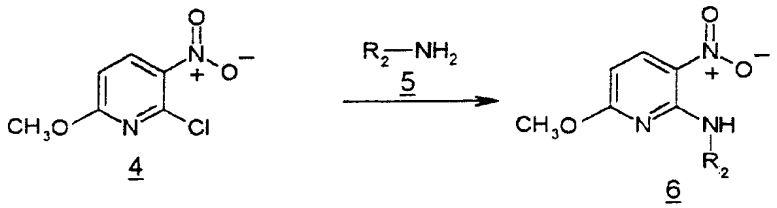


Wherein, X is chloro or methoxy group;

R_2, R_3, R_4 and n are as defined in formula 1.

3. Method for preparation of 6-methoxy-3-nitropyridine derivatives of formula 6 by reacting 2-chloro-6-methoxy-3-nitropyridine derivatives of formula 4 with 5-aminoindazole or 6-aminoindazole of formula 5 in the presence of a base as in scheme 2.

Scheme 2



Wherein, R_2 is as defined in formula 1.

4. Therapeutic agent and a preventive agent for hepatisis B with 3-nitropyridine derivatives and their pharmaceutically acceptable salts in claim 1 as effective ingredient.

5. Therapeutic agent and a preventive agent for acquired immune deficiency syndrome (AIDS) with 3-nitropyridine derivatives and their pharmaceutically acceptable salts in claim 1 as effective ingredient.

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(54) Title: NOVEL 3-NITROPYRIDINE DERIVATIVES AND THE PHARMACEUTICAL COMPOSITIONS CONTAINING SAID DERIVATIVES

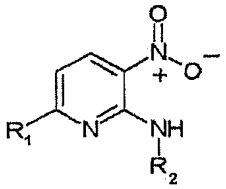
(57) Abstract: The present invention relates to novel 3-nitropyridine derivatives and the pharmaceutical compositions containing said derivatives, and more specifically, to 3-nitropyridine derivatives and their pharmaceutically acceptable salts, the process for preparing them, and the pharmaceutical compositions containing said compounds as active ingredients. In particular, said 3-nitropyridine derivatives of the present invention, due to their inhibitory activity against the proliferation of human immunodeficiency virus (HIV) as well as hepatitis B virus (HBV), can be used as a therapeutic agent as well as a preventive agent for hepatitis B and acquired immune deficiency syndrome (AIDS).

NOVEL 3-NITROPYRIDINE DERIVATIVES AND THE PHARMACEUTICAL COMPOSITIONS CONTANING SAID DERIVATIVES

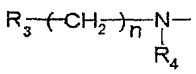
TECHNICAL FIELD

The present invention relates to novel 3-nitropyridine derivatives and the pharmaceutical compositions containing said derivatives. More specifically, the present invention relates to 3-nitropyridine derivatives and their pharmaceutically acceptable salts, represented by the following formula 1, which effectively inhibit proliferation of hepatitis B virus and human immunodeficiency virus. This invention also relates to the process for preparing 3-nitropyridine derivatives and to the pharmaceutical compositions containing said derivatives as effective ingredients against viruses.

Formula 1



Wherein,

R_1 is methoxy or  ;

R_3 is H, hydroxy, dialkylamino group with $C_2 \sim C_6$, straight or branched hydroxyalkyl group with $C_2 \sim C_6$, straight or branched dihydroxyalkyl group with $C_3 \sim C_6$, alkoxyalkyl group with $C_3 \sim C_6$, or saturated or unsaturated 5 or 6 membered heterocyclic compounds containing 1 to 3 heteroatoms selected from N, O, and S, which may be unsubstituted or substituted with alkyl group of $C_1 \sim C_3$; R_3 may or may not contain asymmetrical carbons;

R_4 is H, straight or branched alkyl group with $C_1 \sim C_4$, or cycloalkyl group with $C_3 \sim C_6$;

R_3 and R_4 both may consist of 5 or 6 membered heterocyclic ring containing 1~3 heteroatoms selected from N, O, and S, which is unsubstituted or substituted with straight or branched alkyl group with $C_1 \sim C_5$, straight or branched hydroxyalkyl group with $C_2 \sim C_5$, or hydroxy;

R_2 is indazol-5-yl, or indazol-6-yl;

n is an integer between 0 and 3.

BACKGROUND ART

Hepatitis B virus (HBV; referred as "HBV" hereinafter) causes acute or chronic hepatitis, which may progress to liver cirrhosis and liver cancer. It is estimated that three hundred

million people are infected with HBV in the world (Tiollais & Buendia, *Sci. Am.*, 264, 48, 1991). There has been much research about the molecular biological characteristics of HBV and their relationship to liver diseases in order to find ways to prevent and treat hepatitis B. Various vaccines and diagnostic drugs have been developed and much effort is being channeled into research to find treatment for hepatitis B.

HBV genome consists of genes for polymerase (P), surface protein (pre-S1, pre-S2 and S), core protein (pre-C and C), and X protein. Of these proteins expressed from HBV genes, polymerase, surface protein, and core protein are structural proteins and X protein has a regulatory function.

The gene for HBV polymerase comprises 80% of the whole virus genome and produces a protein of 94kD size with 845 amino acids, which has several functions in the replication of virus genome. This polypeptide includes sequences responsible for activities of protein primer, RNA dependent DNA polymerase, DNA dependent DNA polymerase, and RNase H. Kaplan and his coworkers first discovered reverse transcriptase activities of polymerase, which led to much research in replicating mechanism of HBV.

HBV enters liver when antigenic protein on virion surface is recognized by hepatic cell-specific receptor. Inside the liver cell, DNAs are synthesized by HBV polymerase action, attached to short chain to form complete double helix for HBV genome. Completed double helical DNA genome of HBV produces pre-genomic mRNA and mRNAs of core protein, surface protein, and regulatory protein by the action of RNA polymerase. Using these mRNAs, virus proteins are synthesized. Polymerase has an important function in the production of virus genome, forming a structure called replicasome with core protein and pre-genomic mRNA. This process is called encapsidation. Polymerase has repeated units of glutamic acid at the 3'-end with high affinity for nucleic acids, which is responsible for facile encapsidation. When replicasome is formed, (-) DNA strand is synthesized by reverse transcribing action of HBV polymerase and (+) DNA strand is made through the action of DNA dependent DNA polymerase, which in turn produces pre-genomic mRNAs. The whole process is repeated until the pool of more than 200 to 300 genomes is maintained (Tiollais and Buendia, *Scientific American*, 264: 48-54, 1991).

Although HBV and HIV are different viruses, the replication mechanisms during their proliferation have some common steps, namely, the reverse transcription of virus RNA to form DNA and the removal of RNA strand from subsequently formed RNA-DNA hybrid.

Recently, nucleoside compounds such as lamivudine and famvir have been reported to be useful inhibitors of HBV proliferation, although they have been originally developed as therapeutics for the treatment of acquired immune deficiency syndrome (AIDS; referred as "AIDS" hereinafter) and herpes zoster infection (Gerin, J. L, *Hepatology*, 14: 198-199, 1991; Lok, A. S. P., *J. Viral Hepatitis*, 1: 105-124, 1994; Dienstag, J. L. *et al.*, *New England Journal of Medicine*, 333: 1657-1661, 1995). However, these nucleoside compounds are considered a poor choice for treatment of hepatitis B because of their high cost and side effects such as toxicity, development of resistant virus and recurrence of the disease after stopping treatment. Effort to find therapeutics for hepatitis B among non-nucleoside compounds has been continued and antiviral effects against HBV have been reported for quinolone compounds (EPO 563732, EPO 563734), iridos compounds (KR 94-1886), and terephthalic amide derivatives (KR

96-72384, KR 97-36589, KR 99-5100). In spite of much effort, however, effective drugs for treating hepatitis B have not been developed yet and therapeutic method mainly depends on symptomatic treatment.

AIDS is a disease inducing dramatic decrease in immune function in the body cells and causing various symptoms of infection rarely seen in normal human beings, which spread to the whole body. Human immunodeficiency virus (HIV; referred as "HIV" hereinafter) responsible for AIDS is known to mainly attack helper T cells, which is one of the T cells with regulatory function in the immune system. When helper T cells are infected with HIV virus and undergo necrosis, human immune system cannot function properly. Impairment in immune function subsequently results in fatal infection and development of malignant tumor. Since AIDS patient has been found in USA in 1981 for the first time, the number increased to more than 850,000 patients in 187 countries in 1993 (WHO 1993 report). WHO predicted that 30 to 40 million more people would be infected with HIV by the year 2000 and 10 to 20 million of them would develop the disease.

At the present time, drugs controlling proliferation of HIV have been most widely used for the treatment of AIDS. Of these, Zidovudine, which had been named Azidothymidine previously, is a drug developed in 1987. Didanosine was developed in 1991 as an alternative medicine for AIDS patients when Zidovudine was either ineffective or could not be used due to side effects. In addition, Zalcitabine was approved for concurrent use with Zidovudine in 1992. These drugs alleviate symptoms, slow down progression of the disease in the infected individuals to full-blown AIDS, and somewhat extend life span in the patients. These drugs, however, are not able to cure the patients completely and often develop problems such as resistance and side effects.

In light of these problems, we, inventors of the present invention, tried to develop therapeutics to treat hepatitis B with little chance of toxicity, side effects, and development of resistant viral strains. We found non-nucleoside compounds with excellent antiviral effect against HBV; synthesized novel 3-nitropyridine derivatives represented in formula 1 and completed the invention by showing their dramatic inhibitory

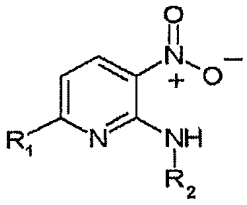
effect on proliferation of HIV as well as of HBV.

DISCLOSURE OF INVENTION

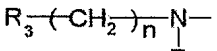
The present invention provides novel 3-nitropyridine derivatives and the pharmaceutical compositions containing said derivatives. More specifically, the present invention provides 3-nitropyridine derivatives and their pharmaceutically acceptable salts, the process for their preparation and the pharmaceutical compositions containing said derivatives as effective ingredient. 3-nitropyridine derivatives of the present invention inhibit proliferation of hepatitis B virus as well as of human immunodeficiency virus and may be effectively used for prevention and treatment of hepatitis B and AIDS.

In order to accomplish the aforementioned goal, the present invention provides novel 3-nitropyridine derivatives represented below in formula 1 and their pharmaceutically acceptable salts.

Formula 1



Wherein,

R_1 is methoxy or  ;

R_3 is H, hydroxy, dialkylamino group with $C_2 \sim C_6$, straight or branched hydroxyalkyl group with $C_2 \sim C_6$, straight or branched dihydroxyalkyl group with $C_3 \sim C_6$, alkoxyalkyl group with $C_3 \sim C_6$, or saturated or unsaturated 5 or 6 membered heterocyclic compounds containing 1 to 3 heteroatoms selected from N, O, and S, which may be unsubstituted or substituted with alkyl group with $C_1 \sim C_3$;

R_3 may or may not have asymmetrical carbons;

R_4 is H, straight or branched alkyl group with $C_1 \sim C_4$, or cycloalkyl group with $C_3 \sim C_6$;

R_3 and R_4 both may consist of 5 or 6 membered heterocyclic ring with 1 to 3 heteroatoms selected from N, O, and S, which is either unsubstituted or substituted with straight or branched alkyl group with $C_1 \sim C_5$, straight or branched hydroxyalkyl group with $C_2 \sim C_5$, or hydroxy;

R_2 is indazol-5-yl, or indazol-6-yl;

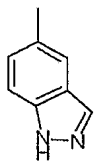
n is an integer between 0 and 3.

When both R_3 and R_4 are represented as a 5 or 6 membered heterocyclic compounds with 1 to 3 heteroatoms selected from N, O, and S, n equals 0. This heterocyclic ring may be unsubstituted or substituted with straight or branched alkyl group with $C_1 \sim C_5$, straight or branched hydroxyalkyl group with $C_2 \sim C_5$, or hydroxy group;

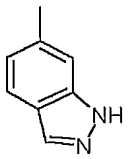
When compounds of formula 1 have asymmetrical carbons, they may exist as either *R* or *S* optical isomer and the present invention covers both optical isomers and the racemic mixture as well.

Indazol-5-yl and indazol-6-yl groups for R_2 in the present invention are represented in formula 2 and 3 respectively.

Formula 2



Formula 3

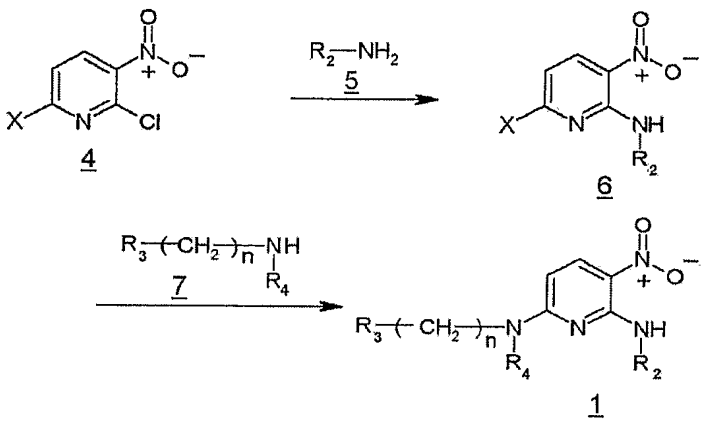


Compounds of formula 1 in the present invention may be utilized in the form of salts and the acid addition salts prepared by adding pharmaceutically acceptable free acids are useful. Compounds of formula 1 may be changed to the corresponding acid addition salts according to the general practices in this field. Both inorganic and organic acids may be used as free acids in this case. Among inorganic acids, hydrochloric acid, hydrobromic acid, sulfuric acid, and phosphoric acid may be used. Among organic acids, citric acid, acetic acid, lactic acid, tartaric acid, maleic acid, fumaric acid, formic acid, propionic acid, oxalic acid, trifluoroacetic acid, benzoic acid, gluconic acid, methanesulfonic acid, glycolic acid, succinic acid, 4-toluenesulfonic acid, galacturonic acid, embonic acid, glutamic acid and aspartic acid may be used.

The present invention also provides the process for preparing 3-nitropyridine derivatives of formula 1 as

represented in scheme 1.

Scheme 1



Wherein, X is Cl or OCH_3; R_2, R_3, R_4 and n are as defined in formula 1.

The process of preparation in the present invention comprises the following steps:

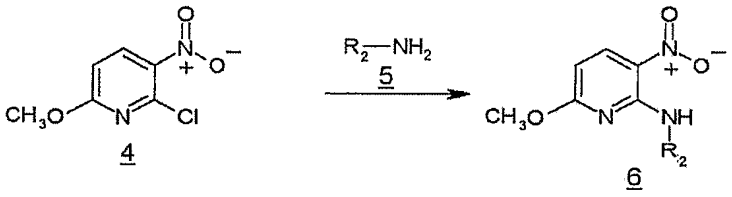
(Step 1) Synthesis of 3-nitropyridine derivatives of formula 6 by reacting 2-chloro-3-nitropyridine derivatives of formula 4 with 5-aminoindazole or 6-aminoindazole of formula 5 in a proper solvent under a basic condition at an appropriate temperature;

(Step 2) Synthesis of 3-nitropyridine derivatives of formula 1 by reacting 3-nitropyridine derivatives of formula

6 prepared in step 1 with appropriate amine compounds of formula 7 in a proper solvent under a basic condition at an appropriate temperature.

When R_1 in the compound of formula 1 is a methoxy group, step 1 completes the synthesis of desired compound ($X=OCH_3$). In this case, the present invention includes the method of preparing 6-methoxy-3-nitropyridine derivatives of formula 6 by reacting 2-chloro-6-methoxy-3-nitropyridine of formula 4 with 5-aminoindazole or 6-aminoindazole of formula 5 in the presence of a base.

Scheme 2



Compound of formula 4 in the first step of scheme 1 is 2-chloro-6-methoxy-3-nitropyridine or 2,6-dichloro-3-nitropyridine.

Chemical reagents used in the first and the second steps of scheme 1, namely, 2-chloro-3-nitropyridine derivatives of

formula 4, 5-aminoindazole or 6-aminoindazole of formula 5, and amine compounds of formula 7, are commercially available and may be purchased.

Compound of formula 7 in the step 2 above is used to introduce a substituent (R_3-$(CH_2)_n$-NR_4-) into the compound of formula 1 and an appropriate amine compound should be selected depending on the substituent desired, which can be easily done by one with general knowledge in the technical field.

To give more specific details about step 1 in the synthetic process, an organic base may be used and common tertiary amines such as triethylamine, *N,N*-diisopropylethylamine, *N*-methylmorpholine, *N*-methylpiperidine, 4-dimethylaminopyridine, *N,N*-dimethylaniline, 2,6-lutidine, pyridine are preferable.

Preferable reaction time and temperature are 4～15 hrs and 20～60 ℃.

Preferable for the reaction is a single or a mixture of solvents selected from chloroform, methylene chloride, acetonitrile and alcohols such as methanol and ethanol.

Of 3-nitropyridine derivatives of formula 6 produced in the reaction of step 1, one with chloro group at 6 position is

used in the following reaction of step 2.

The reaction in step 2 is described in more detail. Preferable solvent is a single or a mixture of solvents selected from acetonitrile, chloroform, methylene chloride, tetrahydrofuran, *N,N*-dimethylformamide, *N*-methylpyrrolidinone, pyridine, water and alcoholic solvents such as methanol, ethanol, and isopropanol.

It is preferable to use excess amount of amine compound (formula 7) to increase the efficiency of the reaction. Solvents used in the previous step 1 for the synthesis of 3-nitropyridine derivatives of formula 6 are preferable. Reaction temperature of 25~80 ℃ is preferable although it depends on the kind of amine compound used.

In another aspect of this invention, also provided are the pharmaceutical compositions of therapeutics for preventing and treating hepatitis B, which contain 3-nitropyridine derivatives of formula 1 and their pharmaceutically acceptable salts as effective ingredients.

The present invention also provides the pharmaceutical

compositions of therapeutics for preventing and treating AIDS, which contain 3-nitropyridine derivatives and their pharmaceutically acceptable salts of formula 1 as effective ingredients.

3-nitropyridine derivatives of formula 1 in this invention have inhibitory effect on proliferation of both HIV and HBV because they interfere with removal of RNA strand from RNA-DNA hybrid formed during the reverse transcription of viral RNA to DNA, which is a common step in the replication mechanism of the two viruses.

Compounds of formula 1 may be taken orally as well as through other routes in clinical uses; for example, it may be administered intravenously, subcutaneously, intraperitoneally, or locally and used in the form of general drugs.

For clinical use of drugs with the pharmaceutical compositions of the present invention, compounds of formula 1 may be mixed with pharmaceutically acceptable excipients and made into various pharmaceutically acceptable forms; for example, tablets, capsules, trochese, solutions, suspensions for oral administration; and injection solutions, suspensions, or dried

powder to be mixed with distilled water for the formulation of instant injection solution.

Effective dosage for compound of formula 1 is generally 10~500 mg/kg, preferably 50~300 mg/kg for adults, which may be divided into several doses, preferably into 1~6 doses per day if deemed appropriate by a doctor or a pharmacist.

BEST MODE FOR CARRYING OUT THE INVENTION

Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

<Preparation Example 1> Preparation of 6-chloro-2-(1*H*-5-indazolylamino)-3-nitropyridine.

To the solution of 2,6-dichloro-3-nitropyridine (4 g) and 5-aminoindazole (2.8 g) in acetonitrile (50 ml) was added triethylamine (3.2 ml), and then the solution was reacted at

20-25℃ for 12 hr. The reaction mixture was cooled at room temperature, added H_2O 30 ml slowly, and then reacted at 20℃ for 1 hr. The reaction mixture was filtered, washed with the mixture solution(15 ml) of acetonitrile : H_2O = 1: 1 (volume ratio) and dried at 50℃ *in vacuo* to obtain the desired compound (4.7 g, 78%).

m.p. : 233 ℃ (dec.)

^{1}H-NMR (DMSO-d_6), ppm : δ 6.94(d, 1H), 7.44(d, 1H), 7.56(d, 1H), 7.91(s, 1H), 8.08(s, 1H), 8.53(d, 1H), 10.20(s, 1H), 13.12(br s, 1H)

<Preparation Example 2> Preparation of 6-chloro-2-(1*H*-6-indazolylamino)-3-nitropyridine.

To the solution of 2,6-dichloro-3-nitropyridine (4 g) and 6-aminoindazole (2.8 g) in acetonitrile (50 ml) was added triethylamine (3.2 ml), and then the solution was reacted at 35-40℃ for 12 hr. The reaction mixture was cooled at room temperature with slowly adding H_2O (20 ml), and then reacted at 20-25℃ for 1 hr. The reaction mixture was filtered, washed with acetonitrile (6 ml) and H_2O (15 ml), and then dried at 50℃ *in vacuo* to obtain the desired compound (4.4 g, 73%).

m.p. : 234 ℃ (dec.)

^{1}H-NMR (DMSO-d_6), ppm : δ 7.02(d, 1H), 7.20(d, 1H), 7.73(d, 1H), 7.99(d, 2H), 8.55(d, 1H), 10.26(s, 1H), 13.09(s, 1H)

<Example 1> Preparation of 2-(1*H*-5-indazolylamino)-6-methoxy-3-nitropyridine.

To the solution of 2-chloro-6-methoxy-3-nitropyridine (5 g) and 5-aminoindazole (3.7 g) in methanol (60 ml) was added triethylamine (4.1 ml), and then the solution was reacted at 25-30 ℃ for 5 hr. The reaction mixture was cooled at room temperature with slowly adding H_2O (30 ml), and then stirred for 0.5 hr. The reaction mixture was filtered, washed with methanol (10 ml), obtained a solid product. The solid product was dried at 50℃ *in vacuo* to obtain the desired compound (6.5 g, 86%).

m.p. : 206~208 ℃

^{1}H-NMR (DMSO-d^6), ppm : δ 3.80(s, 3H), 6.32(d, 1H), 7.55(m, 2H), 8.06(s, 2H), 8.43(d, 1H), 10.52(s, 1H), 13.09(br s, 1H)

<Example 2> Preparation of 2-(1*H*-6-indazolylamino)-6-methoxy-3-nitropyridine.

To the solution of 2-chloro-6-methoxy-3-nitropyridine (5 g) and 6-aminoindazole (3.9 g) in methanol (60 ml) was added triethylamine (4.1 ml), and then the solution was reacted at 55-60℃ for 14 hr. The reaction mixture was cooled at room temperature, added H_2O 30 ml slowly at 25℃, and then stirred for 0.5 hr. The reaction mixture was filtered, washed with 50% aqueous methanol solution (15 ml) and obtained a solid product. The solid product was dried at 50℃ *in vacuo* to obtain the desired compound (6.8 g, 90%).

m.p. : 261~264 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 3.94(s, 3H), 6.39(d, 1H), 7.24(m, 1H), 7.71(d, 1H), 8.01(s, 1H), 8.19(s, 1H), 8.44(d, 1H), 10.62(s, 1H), 13.04(br s, 1H)

<Example 3> Preparation of 2-(1*H*-5-indazolylamino)-6-methylamino-3-nitropyridine.

To the solution of methanol with 40% methylamine (20 ml) was added 2-(1*H*-5-indazolylamino)-6-methoxy-3-nitropyridine (1 g) obtained by the example 1, and the solution was reacted at 25℃ for 1 hr. The reaction mixture was added H_2O (20ml) slowly and stirred for 1 hr. The reaction mixture was filtered, washed

with 30% aqueous methanol solution (5 ml) and then obtained a solid product. The solid product was dried at 50-60 ℃ *in vacuo* to obtain the desired compound (0.82 g, 82%).

m.p. : 238~240 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 2.86(d, 3H), 6.09(d, 1H), 7.51(d, 1H), 7.57(d, 1H), 8.05(t, 2H), 8.24(d, 2H), 10.97(s, 1H), 13.05(br s, 1H)

<Example 4> Preparation of 2-(1*H*-6-indazolylamino)-6-methylamino-3-nitropyridine.

To the solution of methanol with 40% methylamine (20 ml) was added 2-(1*H*-6-indazolylamino)-6-methoxy-3-nitropyridine (1 g) obtained by the example 2, and the solution was reacted at 25-30℃ for 2 hr. The reaction mixture was cooled and stirred at 20℃ for 0.5 hr. The reaction mixture was filtered, washed with methanol (4 ml) and then obtained a solid product. The solid product was dried at 40℃ *in vacuo* to obtain the desired compound (0.79 g, 79%).

m.p. : > 270 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 2.98(d, 3H), 6.15(d, 1H), 7.18(d, 1H), 7.69(d, 1H), 7.99(s, 1H), 8.09(d, 1H), 8.35(br s, 1H), 8.44(s,

1H), 11.14(s, 1H), 13.03(br s, 1H)

<Example 5> Preparation of 2-(1*H*-5-indazolylamino)-6-isopropylamino-3-nitropyridine.

To the solution of 2-(1*H*-5-indazolylamino)-6-methoxy-3-nitropyridine (1 g) obtained by the example 1 in methanol (20 ml) was added isopropylamine (20 ml) slowly and reacted at 45℃ for 20 hr. The reaction mixture was cooled, added H_2O (60 ml) at 25℃ and then stirred for 1 hr. The reaction mixture was filtered, washed with 20 % aqueous methanol solution (5 ml) and then obtained a solid product. The solid product was dried at 50~60℃ *in vacuo* to obtain the desired compound (1.05 g, 96%).

m.p. : 233~235 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 1.15(d, 6H), 4.03(m, 1H), 6.06(d, 1H), 7.50(d, 2H), 8.05(m, 2H), 8.15(t, 2H), 10.97(s, 1H), 13.06(br s, 1H)

<Example 6> Preparation of 2-(1*H*-6-indazolylamino)-6-isopropylamino-3-nitropyridine.

To the solution of

2-(1*H*-6-indazolylamino)-6-methoxy-3-nitropyridine (1 g) obtained by the example 2 in methanol (20 ml) was added isopropylamine (20 ml) slowly and reacted at 45℃ for 45 hr. The reaction mixture was cooled and stirred at 25℃ for 1 hr. The reaction mixture was filtered, washed with methanol (5 ml) and then obtained a solid product. The solid product was dried at 40~50℃ *in vacuo* to obtain the desired compound (0.95 g, 87%).

m.p. : > 270℃

^{1}H-NMR (DMSO-d_6), ppm : δ 1.23(d, 6H), 4.17(m, 1H), 6.12(d, 1H), 7.15(d, 1H), 7.68(d, 1H), 8.00(s, 1H), 8.09(d, 1H), 8.28(d, 1H), 8.35(s, 1H), 11.12(s, 1H), 13.08(br s, 1H)

<Example 7> Preparation of 2-(1*H*-5-indazolylamino)-6-isobuthylamino-3-nitropyridine.

To the solution of 2-(1*H*-5-indazolylamino)-6-methoxy-3-nitropyridine (1 g) obtained by the example 1 in methanol (20 ml) was added isobuthylamine (15 ml) slowly and reacted at 45~50℃ for 20 hr. The reaction mixture was cooled, added H_2O (40 ml) slowly and then stirred at 25℃ for 1 hr. The reaction mixture was filtered, washed with 30% aqueous methanol solution (5 ml), obtained a

solid product. The solid product was dried at 50~60℃ *in vacuo* to obtain the desired compound (0.95 g, 83%).

m.p. : 230~232 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 0.83(d, 6H), 1.83(m, 1H), 3.11(d, 2H), 6.11(d, 1H), 7.50(s, 2H), 7.99(s, 1H), 8.06(d, 1H), 8.19(d, 1H), 8.39(t, 1H), 10.91(s, 1H), 13.07(br s, 1H)

<Example 8> Preparation of 6-cyclopropylamino-2-(1*H*-5-indazolylamino)-3-nitropyridine.

To the solution of 2-(1*H*-5-indazolylamino)-6-methoxy-3-nitropyridine (1 g) obtained by the example 1 in methanol (20 ml) was added cyclopropylamine (10 ml) slowly, heated and reacted at 40~45℃ for 25 hr. The reaction mixture was cooled with adding H_2O (40 ml) slowly and stirred at 25℃ for 1 hr. The reaction mixture was filtered, washed with 30% aqueous methanol solution (5 ml), obtained a solid product. The solid product was dried at 50℃ *in vacuo* to obtain the desired compound (0.82 g, 75%).

m.p. : 237~240 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 0.56(m, 2H), 0.81(m, 2H), 2.81(br s,1H), 6.06(d, 1H), 7.50(d, 1H), 7.62(d, 1H), 8.02(s, 1H), 8.09(d,

1H), 8.46(s, 1H), 8.57(s, 1H), 11.02(s, 1H), 13.04(br s, 1H)

<Example 9> Preparation of 6-amino-2-(1*H*-5-indazolylamino)-3-nitropyridine.

To the solution of 6-chloro-2-(1*H*-5-indazolylamino)-3-nitropyridine (1 g) obtained by the preparation example 1 in chloroform (20 ml) was added 7 N ammonia solution in methanol (30 ml) and reacted at 35~40 ℃ for 15 hr. The reaction mixture was cooled, concentrated under reduced pressure at 25 ℃ and then precipitated with treatment of methanol (10 ml). The reaction mixture was filtered, which was recrystallised with methanol : methylene chloride = 4: 1 to obtain the desired compound (0.63 g, 67%)

m.p. : 263 ℃ (dec.)

^{1}H-NMR (DMSO-d_6), ppm : δ 6.05(d, 1H), 7.48(m, 2H), 7.56(br s, 1H), 7.65(br s, 1H), 8.01(s, 1H), 8.12(d, 1H), 8.28(s, 1H), 10.81(s, 1H), 13.06(br s, 1H)

<Example 10> Preparation of 6-(2-hydroxyethyl)methylamino-2-(1*H*-5-indazolylamino)-3-nitropyridine.

To the solution of 6-chloro-2-(1*H*-5-indazolylamino)-3-nitropyridine (1 g) obtained by the preparation example 1 in acetonitrile (20 ml) was added 2-(methylamino)ethanol (1.4 ml) and triethylamine (0.6 ml) and then refluxed for 12 hr. The reaction mixture was cooled, precipitated with adding excess H_2O at 20~25℃, filtered to obtain solid. The above obtained solid was washed with water, dried at 50℃ *in vacuo* and recrystallised with chloroform : ether = 1: 3 to obtain the desired compound (0.7 g, 62%).

m.p. : 172~174 ℃

^{1}H-NMR (DMSO-d_6), ppm : δ 3.14(s, 3H), 3.64(m, 4H), 4.80(d, 1H), 6.36(d, 1H), 7.50(s, 2H), 8.02(s, 1H), 8.15(m, 2H), 10.71(d, 1H), 13.03(br s, 1H)

It was prepared compounds in prepared example 11~30 as the same method used for the example 10. It is shown in Table 1 that the compound name, yield, recrystallizing solution, melting point of compounds in prepared example 11~30 and 3-nitropyridine derivatives (6) and amine compound (7) as starting materials. It is shown in Table 2 that ^{1}H-NMR of compounds in prepared example 11~30.

TABLE 1a

Example	Compounds				
	3-nitropyridine derivatives (6)	amine compound (7)	Recrystallising soln	Yield (%)	m.p. (℃)
11	6-ethyl-(2-hydroxyethyl)amino-2-(1*H*-5-indazolylamino)-3-nitropyridine				
	Preparation example 1	2-(ethylamino)ethanol	methanol/ether (1:2)	52	164~166
12	6-[(1*S*)-1-(hydroxyethyl)ethylamino]-2-(1*H*-5-indazolylamino)-3-nitropyridine				
	Preparation example 1	(*S*)-2-amino-1-propanol	ethanol	75	>270
13	6-[(1*S*)-1-(hydroxyethyl)ethylamino]-2-(1*H*-6-indazolylamino)-3-nitropyridine				
	Preparation example 2	(*S*)-2-amino-1-propanol	ethanol	69	264~265
14	6-[bis(hydroxymethyl)methylamino]-2-(1*H*-5-indazolylamino)-3-nitropyridine				
	Preparation example 1	2-amino-1,3-propanediol	methanol	80	>270
15	2-(1*H*-5-indazolylamino)-6-(2-methoxy-1-methyl)ethylamino-3-nitropyridine				
	Preparation example 1	2-amino-1-methoxypropane	chloroform/ether (1:5)	58	138~142
16	6-[2-(dimethylamino)ethylamino]-2-(1*H*-6-indazolylamino)-3-nitropyridine				
	Preparation example 2	*N,N*-dimethylethylenediamine	methanol/H_2O (1:2)	84	219~221
17	2-(1*H*-5-indazolylamino)-6-(4-methyl-1-piperazinyl)amino-3-nitropyridine				
	Preparation example 1	1-amino-4-methylpiperazine	mehanol/ether (1:4)	78	170~174
18	2-(1*H*-6-indazolylamino)-6-(4-methyl-1-piperazinyl)amino-3-nitropyridine				
	Preparation example 2	1-amino-4-methylpiperazine	methanol	56	260 (dec.)
19	2-(1*H*-5-indazolylamino)-3-nitro-6-(3-pyridyl)methylaminopyridine				
	Preparation example 1	3-(aminomethyl)pyridine	methanol/H_2O (1:1)	81	250~253
20	2-(1H-6-indazolylamino)-3-nitro-6-(3-pyridyl)methylaminopyridine				
	Preparation example 2	3-(aminomethyl)pyridine	ethanol	77	>270
21	2-(1*H*-5-indazolylamino)-3-nitro-6-(4-pyridyl)methylaminopyridine				
	Preparation example 1	4-(aminomethyl)pyridine	ethanol/H_2O (2:1)	75	219~221
22	2-(1*H*-6-indazolylamino)-3-nitro-6-(2-pyridyl)methylaminopyridine				
	Preparation example 2	2-(aminomethyl)pyridine	methanol/ethanol (1:1)	72	256 (dec.)
23	2-(1*H*-5-indazolylamino)-3-nitro-6-(1-piperazinyl)pyridine				
	Preparation example 1	piperazine	ethanol	78	270 (dec.)

TABLE 1b

Example	Compounds				
	3-nitropyridine derivatives (6)	amine compound (7)	Recrystallising soln	Yield (%)	m.p. (℃)
24	2-(1*H*-6-indazolylamino)-3-nitro-6-(1-piperazinyl)pyridine				
	Preparation example 2	piperazine	ethanol	88	268 (dec.)
25	2-(1*H*-5-indazolylamino)-6-(4-methyl-1-piperazinyl)-3-nitropyridine				
	Preparation example 1	1-methylpiperazine	methylene chloride/isopropyl ether (1:5)	63	116~120
26	2-(1*H*-6-indazolylamino)-6-(4-methyl-1-piperazinyl)-3-nitropyridine				
	Preparation example 2	1-methylpiperazine	ethanol/H_2O (1:1)	60	253 (dec.)
27	6-[4-(2-hydroxyethyl)-1-piperazinyl]-2-(1*H*-6-indazolylamino)-3-nitropyridine				
	Preparation example 2	N-(2-hydroxyethyl)piperazine	ethanol	82	215~217
28	6-(4-hydroxy-1-piperidinyl)-2-(1*H*-5-indazolylamino)-3-nitropyridine				
	Preparation example 1	4-hydroxypiperidine	ethanol	76	235~239
29	6-(4-hydroxy-1-piperidinyl)-2-(1*H*-6-indazolylamino)-3-nitropyridine				
	Preparation example 2	4-hydroxypiperidine	methanol	80	270~272
30	2-(1*H*-5-indazolylamino)-6-(4-morpholinyl)amino-3-nitropyridine				
	Preparation example 1	4-aminomorpholine	methanol	64	>270

TABLE 2a

Example	NMR solution	^{1}H-NMR data (ppm)
11	DMSO-d_6	δ1.09(d, 3H), 3.56(s, 6H), 4.80(d, 1H), 6.35(d, 1H),7.49(s, 2H), 8.01(s, 1H), 8.15(m, 2H), 10.68(s, 1H), 13.03(br s, 1H)
12	DMSO-d_6	δ1.13(d, 3H), 3.43(t, 2H), 4.01(br s, 1H), 4.81(t, 1H), 6.14(d, 1H), 7.51(t, 2H), 8.01(s, 1H), 8.06(d, 1H), 8.13(d, 1H), 8.20(s, 1H), 10.92(s, 1H), 13.03(br s, 1H)
13	DMSO-d_6	δ1.18(d, 3H), 3.51(m, 2H), 4.13(br s, 1H), 4.81(d, 1H), 6.19(d, 1H), 7.20(d, 1H), 7.69(d, 1H), 8.00(s, 1H), 8.08(d, 1H), 8.25(d, 2H), 11.09(br s, 1H), 13.03(br s, 1H)
14	DMSO-d_6	δ3.54(s, 4H), 4.00(s, 1H), 4.76(s, 2H), 6.21(d, 1H), 7.51(m, 2H), 8.00(s, 1H), 8.06(d, 1H), 8.12(d, 1H), 8.23(s, 1H), 10.93(s, 1H), 13.02(br s, 1H)
15	DMSO-d_6	δ1.12(d, 3H), 3.19(s, 3H), 3.26(m, 1H), 3.37(m, 1H), 4.13(br s, 1H), 6.11(d, 1H), 7.50(s, 2H), 8.00(s, 1H), 8.07 (d, 1H), 8.16(t, 2H), 10.86(s, 1H), 13.06(br s, 1H)
16	DMSO-d_6	δ2.11(s, 6H), 2.45(s, 2H), 3.49(s, 2H), 6.21(d, 1H), 7.23(d, 1H), 7.69(d, 1H), 8.00(s, 1H), 8.09(d, 1H), 8.17(s, 1H), 8.28(br s 1H), 11.04(s, 1H), 13.01(br s, 1H)
17	DMSO-d_6	δ2.30(s, 3H), 2.83(br s, 6H), 3.16(s, 2H), 6.49(d, 1H), 7.50(t, 2H), 8.03(s, 1H), 8.25(t, 2H), 9.15(s, 1H), 10.69(s, 1H), 13.06(s, 1H)
18	DMSO-d_6 +TFA-d_1	δ2.80(s, 3H), 2.99(br s,2H), 3.06(br s, 2H), 3.23(br s, 2H), 3.46(br s, 2H), 6.54(br s, 1H), 7.27(d, 1H), 7.71(d, 1H), 8.14(s, 1H), 8.26(br s, 2H)
19	DMSO-d_6	δ4.51(s, 2H), 6.18(m, 1H), 7.26(br s, 1H), 7.38(d, 1H), 7.43(d, 1H), 7.51(d, 1H), 7.93(s, 2H), 8.14(d, 1H), 8.42(s, 2H), 8.77(br s, 1H), 10.80(s, 1H), 13.03(br s, 1H)
20	DMSO-d_6	δ4.62(s, 2H), 6.22(d, 1H), 7.15(d, 1H), 7.27(br s, 1H), 7.64(d, 2H), 8.00(s, 1H), 8.15(s, 2H), 8.42(s, 1H), 8.50(s, 1H), 8.80(br s, 1H), 10.99(s, 1H), 13.01(br s, 1H)
21	DMSO-d_6	δ4.51(s, 2H), 6.23(d, 1H), 7.15(s, 2H), 7.32(m, 2H), 7.85(d, 2H), 8.16(d, 1H), 8.43(m, 2H), 8.79(t, 1H), 10.77(s, 1H), 13.01(br s, 1H)
22	DMSO-d_6	δ4.70(s, 2H), 6.30(d, 1H), 7.15(d, 1H), 7.25(m, 2H), 7.58(d, 1H), 7.66(t, 1H), 7.99(s, 1H), 8.06(s, 1H), 8.15(d, 1H), 8.52(d, 1H), 8.85(br s, 1H), 10.98(s, 1H) 12.98(br s, 1H)
DMSO :dimethylsulfoxide, TFA : trifluoroacetic acid		

TABLE 2b

Example	NMR solution	^{1}H-NMR data (ppm)
23	DMSO-d_6	δ2.70(s, 4H), 3.59(s, 4H), 6.46(d, 1H), 7.51(s, 2H), 7.96(s, 1H), 8.03(s, 1H), 8.16(d, 1H), 10.55(s, 1H), 13.07(br s, 1H)
24	DMSO-d_6	δ2.76(s, 4H), 3.66(s, 4H), 6.51(d, 1H), 7.16(d, 1H), 7.69(d, 1H), 8.00(s, 1H), 8.07(d, 1H), 8.20(d, 1H), 10.82(s, 1H), 13.07(br s, 1H)
25	DMSO-d_6	δ2.17(s, 3H), 2.34(s, 4H), 3.65(s, 4H), 6.48(d, 1H), 7.51(t, 2H), 7.96(d, 1H), 8.04(d, 1H), 8.18(d, 1H), 10.59(s, 1H), 13.07(br s, 1H)
26	DMSO-d_6	δ2.21(s, 3H), 2.40(s, 4H), 3.74(br s, 4H), 6.54(d, 1H), 7.17(d, 1H), 7.70(d, 1H), 8.00(s, 1H), 8.07(d, 1H), 8.22(d, 1H), 10.78(s, 1H), 13.06(br s, 1H)
27	DMSO-d_6+TFA-d_1	δ3.19(t, 4H), 3.59(m, 4H), 3.74(t, 2H), 4.51(br s, 2H), 6.50(d, 1H), 7.17(d, 1H), 7.69(d, 1H), 8.08(d, 2H), 8.28(d, 1H)
28	DMSO-d_6	δ1.35(m, 2H), 1.76(d, 2H), 3.37(d, 2H), 3.75(br s, 1H), 3.99(br s, 2H), 4.78(s, 1H), 6.50(d, 1H), 7.51(s, 2H), 7.98(d, 1H), 8.02(s, 1H), 8.17(d, 1H), 10.61(s, 1H), 13.05(br s, 1H)
29	DMSO-d_6	δ1.42(m, 2H), 1.82(d, 2H), 3.45(t, 2H), 3.79(br s, 1H), 4.06(br s, 2H), 4.82(s, 1H), 6.55(d, 1H), 7.18(d, 1H), 7.69(d, 1H), 8.00(s, 1H), 8.05(s, 1H), 8.19(d, 1H), 10.81(s, 1H), 13.06(s, 1H)
30	DMSO-d_6	δ2.77(s, 4H), 3.68(s, 4H), 6.55(s, 1H), 7.50(t, 2H), 8.02(s, 1H), 8.25(t, 2H), 9.12(s, 1H), 10.67(s, 1H), 13.04(s, 1H)
DMSO :dimethylsulfoxide, TFA : trifluoroacetic acid		

<Experiment 1> Inhibitory effect on the in vitro activities of HBV polymerase in reverse transcription

The following in vitro experiment was performed to determine the effect of the compounds of formula 1 on the activity of HBV

polymerase during reverse transcription.

The present inventors submitted application for a patent concerning HBV polymerase genetically expressed in and separated from *E.coli*, the process of its preparation, and the method to measure the enzyme activities (KR 94-3918, KR 96-33998). In the present experiments HBV polymerase was used which had been expressed in *E. coli* as stated above.

The method used in the present invention to measure in vitro reverse transcribing activities of HBV polymerase is as follows. Basic principles are the same as for ELISA. Nucleotides with biotin or digoxigenin group attached are included as substrates and anti-DIG antibodies attached to peroxidase enzyme recognize the polymerized substrates.

To the wells coated with streptavidin, 20㎕ of HBV polymerase, 20 ㎕ of reaction mixture (10 μM each of DIG-UTP and Biotin-UTP, 46 mM Tris-HCl, 266 mM KCl, 27.5 mM $MgCl_2$, 9.2 mM DTT substrate/primer hybrid), and 20 ㎕ of test compound(added to 1, 0.1, and 0.01 ㎍/㎖) were added and allowed to react at 22℃ for 15 hrs. During this reaction, HBV polymerase catalyzes DNA synthesis and digoxigenin and biotin attached to nucleotides form bonds with streptavidin coated on the bottom of wells. When

the reaction was done, each well was washed with 250 ㎕ of cleaning buffer (pH 7.0) for 30 seconds, which was repeated five times to remove remaining impurities. 200 ㎕ of anti-DIG-POD antibody was added to each well and allowed to react for 1 hr at 37℃, and the wells were washed with cleaning buffer to remove impurities. 200㎕ each of ABTS™, a substrate of peroxidase, was then added and allowed to react at room temperature for 30 min. Absorbance was measured at 405 nm using ELISA reader.

The percentage of reduction in HBV polymerase activities for reverse transcription was calculated using the group without test compound as a control and the results are shown in Table 3.

TABLE 3a Effect on the HBV polymerase activities in reverse transription

Compound	Inhibition activity on HBV-RT (%)		
	1 ㎍/㎖	0.1 ㎍/㎖	0.01 ㎍/㎖
Example 1	85	54	30
Example 2	76	50	12
Example 3	58	47	20
Example 4	60	51	26
Example 5	96	87	53
Example 6	91	76	49
Example 7	95	80	47
Example 8	72	52	38

TABLE 3b. Effect on the HBV polymerase activities in reverse transription

Compound	Inhibition activity on HBV-RT (%)		
	1 ㎍/㎖	0.1 ㎍/㎖	0.01 ㎍/㎖
Example 9	80	59	40
Example 10	78	58	50
Example 11	85	60	35
Example 12	90	56	38
Example 13	66	35	10
Example 14	85	49	30
Example 15	97	68	47
Example 16	80	55	38
Example 17	75	52	41
Example 18	70	43	25
Example 19	91	58	42
Example 20	66	40	21
Example 21	94	65	45
Example 22	81	60	49
Example 23	96	66	51
Example 24	62	43	20
Example 25	88	54	35
Example 26	63	40	18
Example 27	60	38	15
Example 28	55	34	20
Example 29	52	35	10
Example 30	71	49	32

As shown in Table 3, compounds of the present invention have excellent inhibitory effects on the HBV polymerase activities with more than 90% inhibition at the concentration of 1 μg/ml. Moreover, compounds of the present invention are not expected to have problems such as toxicity and development

of resistant viruses as observed in the use of nucleosides and may be applied together with nucleoside compounds due to different mechanisms of action.

In summary, compounds of the present invention effectively reduce the activities of HBV polymerase, inhibit replication and proliferation of HBV and may be useful as therapeutics for prevention and treatment of hepatitis B.

<Experiment 2> Inhibitory effect on the proliferation of HBV in HBV producing cell line

The following experiment was performed to determine inhibitory effects of compounds of formula 1 on the proliferation of HBV producing cell line.

To test for antiviral effect, replication and proliferation of HBV were measured in HepG 2.2.15, a human liver cancer cell line.

The cell concentration was adjusted to 1×10^5 cells/㎖ and 1 ㎖ was added to each well of a 24-well cell culture plate, which was then kept in a culture box for 3 - 4 days at 37℃ under 5% CO_2 until cells grew sufficiently, changing culture medium everyday. When the cells matured sufficiently, the test

compounds were added to the final concentrations of 0.01, 0.1, and 1 ㎍/㎖. One week after the addition of test compounds, the culture solution was centrifuged at 5,000 rpm for 10 min. 25 ㎕ of supernatant was transferred to a new tube and 5 ㎕ of lysis solution [0.54N NaOH, 0.06% NP40]was added to each tube. After keeping the tube at 37℃ for 1 hr, 30 ㎕ of neutralizing solution [0.09N HCl, 0.1M Tris-HCl, pH 7.4] was added as a reaction solution for competitive polymerase chain reaction (PCR).

PCR was performed using genetic sequence of HBV core protein as a matrix. PCR reaction was carried out by adding 1 unit of Taq polymerase enzyme to 25 pmol of each primer, 250 μM dNTP, 5 ㎕ of PCR reaction solution [0.54N NaOH, 0.06% NP40, 0.09N HCl, 0.1M Tris-HCl, pH 7.4].

DNA polymerized by PCR was electrophoresed on Agarose gel and quantitatively analyzed using an image analyzer (Gel Doc 1000, Bio-Rad) in order to evaluate the effect of compounds of the present invention on the reduction of HBV proliferation.

3TC (lamivudine) was used as a positive control at the same concentrations as those of the test compounds. The percentage of reduction in HBV proliferation was calculated using the group without test compound as a control and the results

are represented in Table 4.

TABLE 4 Inhibitory effect on the HBV proliferation

Compound	Inhibition activity on HBV-RT (%)		
	1 μg/mℓ	0.1 μg/mℓ	0.01 μg/mℓ
Example 1	82	45	20
Example 2	72	40	-
Example 3	53	35	-
Example 4	58	40	-
Example 5	94	81	41
Example 6	90	72	33
Example 7	93	75	30
Example 8	65	46	25
Example 11	83	45	20
Example 12	85	48	29
Example 14	80	41	22
Example 15	95	60	36
Example 19	85	51	30
Example 21	90	50	33
Example 23	92	55	35
Example 25	83	46	25
3TC	99	80	48

As shown above in Table 4, non-nucleoside compounds of the present invention have excellent inhibitory effect on the HBV polymerase activities in reverse transcription with more than 80% reduction of HBV proliferation at the concentration of 1 μg/mℓ. Moreover, compounds of the present invention, being non-nucleosides, may not have problems such as toxicity and early

development of resistant virus strains observed in the use of nucleoside substances. It is also expected that compounds of the present invention may be used in parallel with nucleoside compounds since the former act on allosteric binding pockets while the latter act in the domain of polymerase activities.

As described above, compounds of the present invention have excellent inhibitory effect on the HBV polymerase activities important in reverse transcription step of HBV replication. Based on the mechanism, these compounds are able to effectively control HBV proliferation and may be useful as therapeutics for prevention and treatment of hepatitis B.

<Experiment 3> Inhibitory effect on the in vitro HIV enzyme activities in reverse transcription

The following in vitro experiments were done to determine the effect of compounds of formula 1 on the reduction of HIV enzyme activities in reverse transcription.

Non-radioactive reverse transcriptase assay kit (Boehringer Mannheim) was used in the measurement of in vitro transcriptase activities. 20 ㎕ (40 ng) of HIV transcriptase and 20 ㎕ of reaction mixture containing matrix-primer hybrid

poly(A)oligo$(dT)_{15}$, DIG(digoxigenin)-dUTP, biotin-dUTP, and TTP were added to wells coated with streptavidin. Test compounds were also added at the final concentrations of 0.1 and 1 μg/mℓ and allowed to react at 37℃ for 1 hr. At this time, DNA is formed from RNA by the action of HIV reverse transcriptase, forming bonds with streptavidin coated on the bottom of wells because of digoxigenin and biotin moieties attached to nucleotides.

When the reaction was completed, each well was washed with 250 μℓ of cleaning buffer (pH 7.0) for 30 sec. five times to remove remaining impurities. 200 μℓ of anti-DIG-POD antigen was added to each well, allowed to react at 37℃ for 1 hr and washed as above to remove impurities. 200 μℓ of ABTS™, a substrate for peroxidase, was added to each well and allowed to react at room temperature for 30min. Absorbance at 405 nm was then read for each solution using ELISA reader and used for quantitative determination of inhibitory effect on the HIV transcriptase activities. The percentage of reduction in the activities of HIV reverse transcriptase was calculated using the group without test compound as control and the results are represented in Table 5.

TABLE 5 Inhibitory effect on the activities of HIV reverse transcriptase

Compound	Inhibition activity on HBV-RT (%)	
	1 μg/mℓ	0.1 μg/mℓ
Example 1	75	35
Example 4	70	51
Example 5	55	20
Example 6	69	50
Example 7	72	45
Example 11	67	46
Example 12	64	40
Example 14	59	44
Example 16	75	51
Example 20	84	53
Example 21	72	39
Example 27	81	45

As shown above in Table 5, compounds of the present invention have excellent inhibitory effect on the activities of HIV reverse transcriptase, having more than 70% reduction at the concentration of 1 μg/mℓ. Moreover, it is expected that compounds of the present invention, being non-nucleosidic, do not have problems such as toxicity and early development of resistant virus strains observed in the use of nucleoside substances. Furthermore, compounds of the present invention may be used together with nucleoside compounds since the former act on allosteric binding pockets while the latter act in the domain

of polymerase activities.

As described above, compounds of the present invention have excellent inhibitory effect on the HIV enzyme activities in reverse transcription, which is a step in HIV replication. Based on the mechanism, these compounds are able to effectively control HIV proliferation and may be useful as therapeutics for prevention and treatment of AIDS.

<Experiment 4> Cytotoxicity test

To determine if compounds of formula 1 exhibit cytotoxicity, in vitro tests were carried out using HepG2 cells with MTT analysis method as generally known and the results are in Table 6 shown below.

TABLE 6 Cytotoxicity tests on HepG2 cells

Compound	Cytotoxicity on HepG2	
	IC_{50}[1)]	MCD[2)]
Example 2	>100	100
Example 6	>100	100
Example 11	>100	100

1) IC_{50}: 50% Inhibitory Concentration (㎍/㎖)

2) MCD: Minimal Cytotoxic Concentration (㎍/㎖)

As shown above in Table 6, compounds used in the experiments

have higher than 100 $\mu g/m\ell$ for IC_{50} and are considered to have little cytotoxicity.

<Experiment 5> Acute toxicity in rats tested via oral administration

The following experiments were performed to see if compounds of formula 1 have acute toxicity in rats.

6-week old SPF SD line rats were used in the tests for acute toxicity. Compounds in the examples of 1~22 were suspended in 0.5% methylcellulose solution and orally administered once to 6 rats per group at the dosage of 2 g/kg/15mℓ. Death, clinical symptoms, and weight change in rats were observed, hematological tests and biochemical tests of blood performed, and any abnormal signs in the gastrointestinal organs of chest and abdomen checked with eyes during autopsy. The results showed that the test compounds did not cause any specific clinical symptoms, weight change, or death in rats. No change was observed in hematological tests, biochemical tests of blood, and autopsy. The compounds used in this experiment are evaluated to be safe substances since they do not cause any toxic change in rats up to the level of 2 g/kg and their estimated LD_{50} values are much

greater than 2 g/kg in rats.

INDUSTRIAL APPLICABILITY

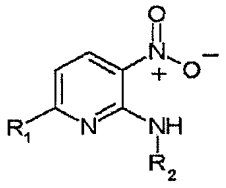
As described above, novel 3-nitropyridine derivatives of formula 1 in the present invention have dramatic inhibitory effect on proliferation of HBV and of HIV with little side effect and may be useful as therapeutic agents for prevention and treatment of hepatitis B and AIDS.

Moreover, it is expected that compounds of the present invention, being non-nucleosidic, do not have problems such as toxicity and early development of resistant virus strains observed in the use of nucleoside substances. Furthermore, compounds of the present invention may be used together with nucleoside compounds since the former seem to act on allosteric binding pockets while the latter work in the domain of polymerase activities.

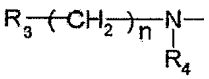
WHAT IS CLAIMED IS;

1. 3-Nitropyridine derivatives and their pharmaceutically acceptable salts as represented by formula 1.

Formula 1



Wherein,

R_1 is methoxy or  ;

R_3 is H, hydroxy, dialkylamino group with $C_2 \sim C_6$, straight or branched hydroxyalkyl group with $C_2 \sim C_6$, straight or branched dihydroxyalkyl group with $C_3 \sim C_6$, alkoxyalkyl group with $C_3 \sim C_6$, or saturated or unsaturated heterocyclic compounds containing 1 to 3 heteroatoms selected from N, O, and S, which may be unsubstituted or substituted with alkyl group of $C_1 \sim C_3$; R_3 may or may not contain asymmetrical carbons;

R_4 is H, straight or branched alkyl group with $C_1 \sim C_4$, or cycloalkyl group with $C_3 \sim C_6$;

R_3 and R_4 both may consist of 5 or 6 membered heterocyclic ring containing 1~3 heteroatoms selected from N, O, and S, which

is unsubstituted or substituted with straight or branched alkyl group with $C_1 \sim C_5$, straight or branched hydroxyalkyl group with $C_2 \sim C_5$, or hydroxy;

R_2 is indazol-5-yl, or indazol-6-yl;

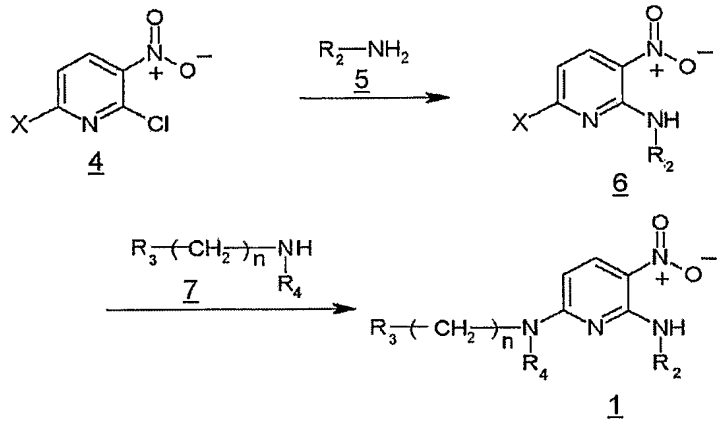
n is an integer between 0 and 3.

2. Process for the preparation of 3-nitropyridine derivatives of formula 1 comprising the following two steps as represented in scheme 1:

Step 1. Synthesis of 3-nitropyridine derivatives of formula 6 by reacting 2-chloro-3-nitropyridine derivatives of formula 4 with 5-aminoindazole or 6-aminoindazole of formula 5 in the presence of a base;

Step 2. Preparation of 3-nitropyridine derivatives of formula 1 by reacting 3-nitropyridine derivatives of formula 6 synthesized in step 1 with amine compounds of formula 7

Scheme 1

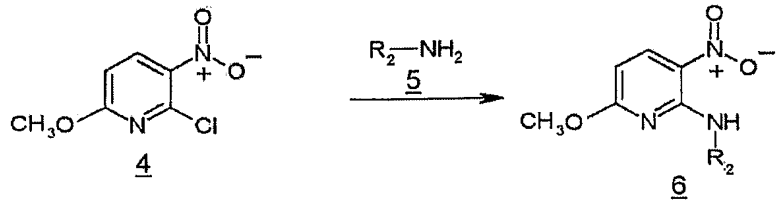


Wherein, X is chloro or methoxy group;

R_2, R_3, R_4 and n are as defined in formula 1.

3. Method for preparation of 6-methoxy-3-nitropyridine derivatives of formula 6 by reacting 2-chloro-6-methoxy-3-nitropyridine derivatives of formula 4 with 5-aminoindazole or 6-aminoindazole of formula 5 in the presence of a base as in scheme 2.

Scheme 2



Wherein, R_2 is as defined in formula 1.

4. Therapeutic agent and a preventive agent for hepatisis B with 3-nitropyridine derivatives and their pharmaceutically acceptable salts in claim 1 as effective ingredient.

5. Therapeutic agent and a preventive agent for acquired immune deficiency syndrome (AIDS) with 3-nitropyridine derivatives and their pharmaceutically acceptable salts in claim 1 as effective ingredient.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR00/01365

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C07D 213/16

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)

IPC7 C07D 213/16

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched

Korean Patents and applications for inventions since 1975
Korean Utility models and applications Utility models for since 1975

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)

CAPLUS(STN), MEDILINE(STN), USPATFULL, NPS, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 563732 A1 (BAYER AG) 6 OCTOBER 1993	1 - 5
A	KR 98-53296 A (DONG WHA PHARM IND. CO. , LTD) 25 SEPTEMBER 1998	1 - 5

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevence

"E" earlier application or patent but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art

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Date of the actual completion of the international search	Date of mailing of the international search report
27 MARCH 2001 (27.03.2001)	28 MARCH 2001 (28.03.2001)

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